EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

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

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Rome, 1–10 February 1994

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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. 41/6, 1994.

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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 FAO headquarters, Rome, from 1 to 10 February 1994. The meeting was opened by Mr F. B. Zenny, Assistant to the Assistant Director-General, Economic and Social Policy Department, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization.

Mr Zenny noted that the scientific integrity and independence of the Committee were its greatest assets. Members of the Committee, invited in their personal capacity with the agreement of their national governments, provided advice and opinion which were respected by all Member States of FAO and WHO. The Committee’s conclusions and recommendations formed a solid basis for the work of the Codex Alimentarius Commission in corresponding areas and were referred to by scientists, food technologists and food officials in governments, universities and industry around the world. The importance of the Committee’s advice to the Codex Alimentarius Commission was further increased by the recent GATT (General Agreement on Tariffs and Trade) Agreement on Sanitary and Phytosanitary Measures, which included Codex standards, codes of practice and maximum residue limits for reference purposes in the case of disputes between trading partners.

Mr Zenny also noted that the Joint FAO/WHO/GATT Conference on Food Standards, Chemicals in Food and Food Trade, held in Rome during March 1991, had discussed in considerable detail both the work and the role of the Committee in establishing a scientific basis for recommended international safety standards for food additives and other substances occurring in food. Among other matters, the Conference recognized the importance of the Committee in providing evaluations based on sound science and on health, safety and technical concerns, and the need for it not to be involved in socioeconomic or ethical issues. He pointed out that the Committee’s deliberations on all issues, in addition to serving as a source of advice to FAO, WHO and their Member States, were also of interest to consumer organizations and non-expert bodies, and should be explained to them as soon, and in as much detail, as possible.

Five previous meetings of the Committee had been held to consider veterinary drug residues in food (Annex 1, references 80, 85, 91, 97 and 104) in response to the recommendations of the Joint FAO/WHO Expert Consultation held in 1984 (1). The present meeting1 was convened in response to the recommendation made at the fortieth meeting of the

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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 41 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
Committee that meetings on this subject should be held annually (Annex 1, reference 104). The Committee’s purpose was to provide guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on public health issues pertaining to residues of veterinary drugs in foods of animal origin. The specific tasks before the Committee were:

- to further elaborate principles for evaluating the safety of residues of veterinary drugs in food and for establishing Acceptable Daily Intakes (ADIs) and Maximum Residue Limits (MRLs) for such residues when the drugs under consideration are administered to food-producing animals in accordance with good practice in the use of veterinary drugs (see section 2);
- to evaluate the safety of residues of certain veterinary drugs (see section 3 and Annex 2); and
- to discuss matters of interest arising from the report of the Twentieth Session of the Codex Alimentarius Commission (section 2.1) and to note the conclusions of the Seventh Session of the Codex Committee on Residues of Veterinary Drugs in Foods (2).

2. General considerations

2.1 Assessment procedures used by the Committee

The Codex Alimentarius Commission, at its Twentieth Session, considered a working paper on risk assessment procedures (3). In presenting the working paper (4), the author noted that the expert committees that advise the Commission form a bridge between those who carry out scientific research and the risk managers (usually Codex Committees), and are ideally suited to undertake risk assessment. In the case of residues of veterinary drugs, it was noted that risk management decisions were an explicit part of the scientific review.

The author of the working paper also noted that the expert committees need to develop risk assessment criteria for the scientific review process and explicitly characterize the uncertainties inherent in safety evaluations; that risk management decisions currently made by the expert committees need to be reassessed; and that an interactive framework should be developed for all policy decisions concerning risk assessment. There is a strong need to promote the use of formal quantitative exposure assessments as part of risk assessment, for example in relation to the dietary intake of foods by particular ethnic and risk groups.

In its discussion of the working paper, the Commission stressed the importance of recognizing the different components of risk assessment and of increasing transparency in the work of the Joint FAO/WHO Expert Committee on Food Additives in, for example, the identification of hazards and the choice of the safety factors to be used in the evaluations. Several delegations recommended that greater attention should be given
to quantifying uncertainty in specific risk assessments carried out by expert committees. The Commission recommended that the paper should be brought to the attention of the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues.

The working paper considered by the Commission clearly defined the various components of risk assessment used in it. However, risk assessment is defined differently in different parts of the world, which creates problems in understanding the roles of various groups in developing food standards. The Committee recommended that a body such as the International Programme on Chemical Safety (IPCS) should continue to coordinate efforts aimed at developing a consensus in this area.

Since its thirty-second meeting in 1987 (Annex 1, reference 80), which was the first to be exclusively devoted to veterinary drugs, the Committee has attempted to describe in detail the data that have been reviewed and the basis for its evaluations. The safety factors applied to no-observed-effect levels (NOELs) in allocating ADIs reflect the uncertainty inherent in the assessments. In some cases the choice of a safety factor higher than the normal 100 reflects an increased uncertainty due to lack of information. In other cases, the safety factor is increased because a specific hazard has been identified (see Annex 1, reference 97, section 2.2). It is difficult to develop more precise quantitative guidelines for establishing safety factors.

In determining MRLs from an ADI the Committee does follow several specific guidelines and procedures. Examples include the decision-tree approach elaborated at the thirty-sixth meeting of the Committee (Annex 1, reference 91), consideration of food factors and intake data (Annex 1, reference 85), the procedure for evaluating the contribution of bound residues (Annex 1, reference 91), and the requirements for residue data, including studies with labelled and unlabelled compound to determine total residues and an appropriate marker residue for use in control procedures. The Committee also requires that analytical methods be readily available to measure residues in food products.

The assessment procedures used by the Committee are continually evolving, those currently adopted reflecting both the dynamic nature of scientific knowledge and assessment procedures, and the greater experience of the Committee as a result of assessing a continually increasing range of veterinary drugs. The assessment of microbiological safety is a good example. The scientific principles used by the Committee in its assessments are summarized in its reports. It is important to recognize that, even with very specific scientific principles to guide the assessments, each compound must be assessed on its own merits. Scientific principles are most important for maintaining consistency in assessments. This is very difficult to achieve in some cases, however, because of the differing pharmacological and toxicological properties of drugs and the widely varying amounts of information available on them.
This latter point was recognized at the fortieth meeting of the Committee (Annex 1, reference 104), when it was concluded that manufacturers of drugs should provide evaluation reports addressing issues relevant to human food safety when the usual studies are not available. The Committee considered that, in such cases, a long history of drug use and data from such use constitute an important factor in establishing the ADI and recommending MRLs. This approach to evaluating drugs with a long history of use was applied both to compounds on the agenda of the fortieth meeting as well as to appropriate compounds at the present meeting.

The daily food intake values considered when recommending MRLs, namely 300 g of meat as muscle tissue, 100 g of liver, 50 g of kidney, 50 g of tissue fat, 100 g of egg, and 1.5 l of milk, undoubtedly protect the vast majority of the population of the world. The value for milk appears particularly high, but the Committee considered this value to be appropriate since it would ensure that young children do not consume residues of veterinary drugs at levels that exceed the ADI on a per kg of body weight basis. While the Committee recognized the need for better dietary intake data for use in assessing the risk of chemicals in general, this is not necessarily the case for residues of veterinary drugs in foods, as stated in the report of the fortieth meeting (Annex 1, reference 104). Nevertheless, governments should consider whether local diets may result in intakes that exceed the ADI.

The Committee was aware that, in a few instances, consumption of food containing drug residues at the recommended MRL could result in a residue intake marginally exceeding the ADI. However, the Committee considered this to be a very rare occurrence, and that overall, the approach provides a more than adequate margin of safety for the consumption of the products under consideration.

In view of the long period of time that often elapses between assessments by the Committee and the adoption of recommended MRLs by the Codex Alimentarius Commission, the Committee welcomed the accelerated procedure for veterinary drug residues that has been adopted by the Commission.

2.2 Assessment of microbiological risk due to residues of antimicrobial drugs in food

The need to evaluate the microbiological risk associated with residues of antimicrobial animal drugs was discussed at the thirty-sixth and thirty-eighth meetings of the Committee (Annex 1, references 91 and 97). At the thirty-sixth meeting, the Committee stated that “in the absence of in vivo data, in vitro data such as minimum inhibitory concentrations may be used, on a temporary basis, for safety evaluations” (emphasis added).

At its present meeting, the Committee still considered that antimicrobial activity may be an appropriate end-point for establishing the ADI for
antimicrobial animal drug residues. However, the Committee was also aware of evidence that the microbiological risk associated with exposure to the very low levels of antimicrobial drugs present as residues in food is minimal, and that other methods for examining microbiological endpoints are under development. The application of these new methods may assist the Committee in assessing the microbiological risk of antimicrobial drug residues in food. It therefore considered that it should remain flexible in its approach to establishing an ADI for the residue of an antimicrobial drug. Until there is more general agreement on the appropriateness of different methods of assessing microbiological risk, the Committee may accept extensive in vitro studies of the antimicrobial activity on human intestinal bacteria as an alternative to more direct evidence that residue intake will not have an adverse effect on human intestinal ecology. In the meantime, the resulting ADI may be either temporary or final, depending on both the quality and quantity of the information supplied (see spectinomycin, section 3.2.4).

2.3 Residues at the injection site

At its thirty-eighth meeting (Annex 1, reference 97), the Committee expressed its concern regarding the possible high concentrations of drug residues at injection sites. In some instances these injection sites might not be detected during inspection procedures and may be in non-discarded edible tissues. This matter was addressed during the Committee’s deliberations on some of the compounds reviewed at the present meeting, where particular attention was drawn to the drugs concerned (e.g., dexamethasone). The problem arises in relation to the parent drug in long-acting formulations and to products that, on hydrolysis, yield the parent drug, possibly resulting in persistent residues at the injection site. It would be helpful at future meetings to have additional pharmacokinetic information on veterinary drugs that may be hydrolysed to parent drug.

If the principles outlined in the report of the thirty-eighth meeting (Annex 1, reference 97) are applied, however, the Committee reaffirmed that there will be no hazard associated with the consumption of residues from the injection site.

2.4 Relevant data for assessing the human food safety of residues of veterinary drugs

When investigating the safety of the consumption of residues of veterinary drugs in food, the Committee requires detailed reports (including individual animal data) of the following types of studies relevant to the toxicological evaluation:

- Pharmacokinetic, metabolic and pharmacodynamic studies in experimental and food-producing animals, and in humans, when available.
- Short-term, long-term/carcinogenicity, reproduction and developmental studies in experimental animals, and genotoxicity studies.
Special studies designed to investigate specific effects, such as those on mechanisms of toxicity, no-hormonal-effect levels, immune responses or macromolecular binding.

For compounds with antimicrobial activity, studies by the manufacturer designed to evaluate the possibility that the compound might have an adverse effect on the microbial ecology of the human intestinal tract.

Studies providing relevant data on the use of, and exposure to, the drug in humans, including studies of effects observed after occupational exposure and epidemiological data following clinical use in humans.

Detailed reports of studies relevant to the evaluation of drug residues in food-producing animals that are required for evaluation should include information on:

- The chemical identity and properties of the drug.
- Its use and dose range.
- As for the toxicological evaluation, pharmacokinetic and metabolic studies in experimental animals, target animals and humans, when available.
- Residue-depletion studies with radiolabelled drug in target animals from zero withdrawal time to periods extending beyond the recommended withdrawal time. These studies should provide information on total residues, including free and bound residues, and major residue components to permit selection of a marker residue and target tissue.
- Residue-depletion studies with unlabelled drug for the analysis of marker residue in target animals and in eggs, milk and honey. These should include studies with appropriate formulations, routes of application, and species, at doses up to the maximum recommended.
- A review of routine analytical methods that may be used by regulatory authorities for the detection of residues in target tissue.
- A description of the analytical procedures used by the sponsor for the detection and determination of parent drug residues. The sponsor is also required to describe a method that may be used by regulatory authorities for the specific determination of the marker residue with a sensitivity equal to or less than the MRL.

In addition, studies designed to assess the impact of residues of antimicrobial agents on food processing, e.g. cheese and yoghurt production, may be required (Annex 1, reference 97, section 2.4.2).

This list is not intended to be exhaustive, and it is recognized that other studies may, in some instances, be of assistance in the evaluation.

As already mentioned, the Committee has established procedures for the evaluation of veterinary drugs with a long history of use (Annex 1, reference 104). In particular, when all of the areas of concern listed above are not addressed by adequate studies, the manufacturer is requested to provide an evaluation report addressing issues relevant to human food safety.
The Committee requests from manufacturers copies of all reports, both positive and negative, that will assist in the assessment, and requires that data be presented, summarized and referenced in a clear and concise manner. When the manufacturer is in doubt about the relevance of studies, the Committee recommends that they should be included in the submission.

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

The Committee considered for the first time two antimicrobial agents and one glucocorticosteroid. It reconsidered one anthelminthic agent, three antimicrobial agents and one trypanocide. New information was not available on one antiprotozoal agent, so the temporary ADI was not extended. The recommendations made with regard to these compounds are summarized in Annex 2.

Toxicological monographs were prepared for all of the substances considered in this section except ronidazole. Residues monographs were prepared on all substances except flumequine and ronidazole.

3.1 **Anthelminthic agent**

3.1.1 **Levamisole**

Levamisole has a long history of use as a broad-spectrum anthelminthic in animals. It is used in human medicine as an anthelminthic and immunomodulator. Levamisole had previously been evaluated at the thirty-sixth meeting of the Committee (Annex 1, reference 97). A temporary ADI of 0–0.003 mg per kg of body weight was established based on a NOEL of 1.25 mg per kg of body weight per day for haemolysis in dogs and a safety factor of 500.

**Toxicological data**

Information from a limited number of studies was submitted for consideration at the present meeting, including data from pharmacokinetic and metabolism studies, special studies on haematological and immunological effects in dogs, and results following administration to humans.

The *in vitro* biotransformation of levamisole was investigated using hepatocytes and liver microsomes from dogs, pigs, sheep, cattle and humans. The results indicated qualitatively similar degradation pathways in each species. Following oral dosing in animals, similar metabolic pathways were identified in rats, dogs, monkeys and cattle, confirming previously reviewed data in rats. Characterization of human metabolites
was limited, but the available evidence indicates similar pathways to those in other species. All metabolites identified in cattle were also observed in dogs and rats, and the toxicological potential of beef residues may therefore be considered to have been evaluated in laboratory animal studies.

Pharmacokinetic studies in humans revealed that peak plasma levels were achieved 1–2 hours after an oral dose, the levels being proportional to the dose given. The metabolism of levamisole was both extensive and rapid, but the metabolites were eliminated more slowly than the parent drug. Excretion was mainly in the urine.

In two repeat-dose toxicity studies in dogs, in which an induction–challenge dosing regimen was used, some of the levamisole-treated animals developed haemolytic anaemia. This confirmed the susceptibility of the species to induction of haemolytic anaemia by levamisole, as noted in the Committee’s thirty-sixth report (Annex 1, reference 91). Red cell parameters returned to normal on cessation of dosing, but anaemia quickly returned in most dogs when treatment was recommenced. Additionally, thrombocytopenia and leukopenia were induced at incidences lower than that of haemolytic anaemia but with a similar dose–response relationship. The levamisole-induced incidence of granulocytopenia in both humans and dogs is low.

The haemolytic anaemia and leukopenia were severe enough to necessitate cessation of dosing in many animals, and resulted in the death of a number of dogs. The induction dose was 20 mg per kg of body weight per day but, in sensitized animals, challenge with doses of 1.25 mg per kg of body weight per day and above produced a dose-related incidence of the re-emergence of haemolytic anaemia. None the less, a previous study in dogs showed no haematological toxicity at a dose of 1.25 mg per kg of body weight per day given continuously for a period of 1 year.

In one study in dogs, plasma levels of levamisole increased in proportion to the dose, but there was no clear correlation between haematological toxicity and plasma drug level. However, the metabolites that may play a role in the induction of anaemia were not measured.

Various immunological parameters were investigated in the two studies in dogs, with a view to elucidating the mechanism underlying the haematological effects. Sera obtained from dogs sensitized to levamisole caused the agglutination of erythrocytes from an untreated dog. The agglutination response was enhanced in the presence of levamisole or some of its metabolites, but only in three of 24 animals studied. In erythrocytes isolated from sensitized animals, IgM antibodies and complement were present on cell surfaces during periods of levamisole-induced haemolytic anaemia. IgG antibodies did not correlate well with anaemia in dogs.

Sera from humans treated with levamisole and showing severe leukopenia or agranulocytosis caused leukocyte agglutination or complement-
dependent granulocytotoxicity *in vitro*. The factors responsible for these effects were strongly correlated with haematological toxicity, while sera from patients not developing agranulocytosis were not toxic to normal white blood cells. Analysis of sera from a limited number of individuals revealed the presence of IgM, but not IgG. Leukocyte agglutination was dependent on the presence of levamisole, but granulocytotoxicity was not.

Although the primary target cells in humans and dogs are generally different, there is now evidence supporting an immunological basis for the haematological toxicity observed in both species. This implicates the involvement of IgM antibodies and a dependence on complement in the mechanism of cellular destruction. There is also limited evidence that agglutination responses in humans and dogs are mediated through antiphagocytosis antibodies, possibly induced by immunogenic complexes between levamisole and protein, to which the drug is known to bind. The reasons for the differential cell sensitivity in humans and dogs are not known; however, the similarities in etiology and the recent demonstration of leukopenia in dogs suggest that these animals are a suitable model for the haematological toxicity of levamisole in humans.

The Committee noted that the further studies reviewed at the present meeting provided information on the incidence of, and mechanism underlying, the haematological effects in humans and dogs. It also noted that, as previously mentioned, continuous dosing of dogs with 1.25 mg per kg of body weight per day of levamisole for a period of 1 year did not result in haemolytic anaemia, but that this dose did cause the re-emergence of haemolytic anaemia in a number of dogs previously sensitized with 20 mg per kg of body weight per day of levamisole. Since a very small number of humans are sensitized to levamisole following therapeutic exposure, the Committee considered that the use of a safety factor of 200 would be appropriate. On this basis, an ADI of 0–6 μg per kg of body weight was established.

*Residue data*

Levamisole, the laevorotatory isomer of tetramisole, is readily absorbed, independently of the route of administration, and rapidly excreted, predominantly in the urine. With time, an increasing fraction of the dose remaining in the animal is bound to larger molecules, forming non-extractable residues.

Levamisole is rapidly metabolized to a large number of metabolites, of which some of the major ones have been identified. Information on metabolism in *in vivo* studies has been reported for cattle, dogs, rats and monkeys. The sponsors carried out a new definitive study in which 14C-labelled levamisole was administered to cattle; this provided new information on the metabolites of levamisole in urine, plasma and liver. Metabolism in other farm animal species was investigated in *in vitro* studies in liver.
The results of all the in vivo and in vitro studies in target species and laboratory animals indicate that the routes of metabolism of levamisole are qualitatively similar in most species.

In the new study of [14C]levamisole in cattle, the metabolites of levamisole in urine, plasma and liver were investigated. There was ample evidence of extensive and rapid metabolism of levamisole. Only 0.4-2.3% of the dose was present in urine or faeces as unchanged drug, which also only accounted for less than 1.3-3.6% of the total residues in tissues.

There appear to be two main metabolic pathways:

1. Oxidation at the 2 position of the imidazothiazole ring followed by oxidation to a carbonyl and hydrolysis to a thiohydantoic acid (R 92535). This in turn is further metabolized to a glucuronyl conjugate (M7) or may lose the acid side-chain, or the sulfur group may be replaced by oxygen. A metabolic profile of total urine collected from cattle for 3 days after an intramuscular injection of levamisole showed that R 92535 accounts for 25-47% and M7 5-23% of urinary metabolites.

2. Hydrolysis of the thiazoline ring to yield a mercaptoethyl intermediate which forms the S-cysteinyl-glycine polar conjugate and is found in liver 3 days after dosing.

A new comprehensive radiometric study in cattle was reported which provided useful information for the evaluation of the total residues, the non-extractable residues, a marker compound and the setting of suitable MRLs. Urine and faeces were collected, as were tissue samples when the animals were slaughtered at 3, 7, 14 and 21 days after dosing. The total radioactivity in the tissues was determined, the highest concentrations of residues being found in the liver and kidney. The nature of the residues was investigated further in the liver samples by determining the amount of non-extractable residues and identifying and quantifying the major metabolites in the free fraction. Two major metabolites were present in liver, namely the S-cysteinyl-glycine conjugate noted above and an unidentified metabolite.

The concentration of parent drug measured radiometrically was below the determination limit of the assay. It was therefore measured by means of the more sensitive gas chromatography assay for the unlabelled drug. From this it can be concluded that, over a 14-day period, the unchanged drug is a small (1.3-3.6%, mean 2.4% ± SD 0.7%) but constant proportion of the total residues; other metabolites do not have a linear log relationship with total residues.

The sponsors submitted new studies in which the detection limit for levamisole was 5 or 10 µg/kg. Several old and new studies using a number of different formulations, dose rates and routes of administration were reported in which the concentrations of levamisole in liver of cattle, sheep, swine and poultry were determined. The residues were sometimes less than 100 µg/kg 7 days after dosing, but could be greater than 10 µg/kg at 14 days, and less than 10 µg/kg at 21 days. In cattle given doses greater
than 8 mg per kg of body weight, most of the values were above 10 μg/kg for at least 14 days, and some were above 100 μg/kg at 14 days.

The non-extractable residues form about 50% of the total residues and, from new information, the bioavailability is estimated to be about 15%.

In poultry given twice the recommended dose, residues of parent drug were not measurable in the edible tissues after a 1-day withdrawal period. When poultry were given the recommended dose, the residues in yolks of eggs at 1-day withdrawal are about 800 μg/kg.

Good analytical methods were available for quantifying the parent drug at a concentration of 10 μg/kg in animal tissues.

Maximum Residue Limits
Based on the ADI of 0-6 μg per kg of body weight for parent drug established by the Committee, the permitted daily intake of parent drug and/or its equivalents is 360 μg for a 60-kg person.

The following factors were taken into account in estimating the MRLs:

- The ADI.
- The parent drug is a suitable marker residue and accounts for 2.4% of the total residues.
- All of the residues in muscle and fat are free residues and are equivalent to parent drug.
- Of the residues in liver, 50% are present in the bound fraction, and 15% of the bound residues are bioavailable.
- The residues in kidney are qualitatively similar to those in liver.
- It is assumed that all bioavailable residues in liver and kidney are equivalent to parent drug.
- The residues are similar in cattle, sheep and pigs.

The Committee recommended MRLs in cattle, sheep, pigs and poultry of 10 μg/kg for muscle, kidney and fat, and 100 μg/kg for liver, expressed as parent drug. Because residues at 1-day withdrawal in yolks of eggs laid by hens given the recommended dose were approximately 800 μg/kg, the Committee considered that levamisole should not be used in laying hens, and did not recommend an MRL for eggs. No new data were submitted by the sponsors to support the re-evaluation of levamisole residues in milk. Therefore, the Committee withdrew the temporary MRL for milk allocated at its thirty-sixth meeting (Annex 1, reference 97). The sponsors recommend that levamisole should not be used in lactating cows.

If the recommended values are used for the MRLs and account is taken of the factors mentioned above, the theoretical maximum intake of residue of parent drug and its equivalents is 397 μg per day, of which only 14 μg is parent drug (see Table 1).

In view of the inherent variability involved in estimating total levamisole-equivalent residues based on levamisole as the marker residue and the fact that only a small proportion of the total residues is used to estimate
Table 1
Theoretical maximum daily intake of levamisole residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MRL (µg/kg)</th>
<th>Parent drug (µg)</th>
<th>Free residue (µg levamisole equivalents)</th>
<th>Bioavailable bound residue (µg levamisole equivalents)</th>
<th>Total bioavailable residue (µg levamisole equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>10</td>
<td>3</td>
<td>125</td>
<td>0</td>
<td>125</td>
</tr>
<tr>
<td>Fat</td>
<td>10</td>
<td>0.5</td>
<td>21</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>10</td>
<td>208</td>
<td>31</td>
<td>239</td>
</tr>
<tr>
<td>Kidney</td>
<td>10</td>
<td>0.5</td>
<td>10</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td>397</td>
</tr>
</tbody>
</table>

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

b 15% of the bound fraction.

the total levamisole equivalents, the Committee considered this value to be approximately the same as the ADI.

3.2 Antimicrobial agents

3.2.1 Chloramphenicol

Chloramphenicol is a broad-spectrum antibiotic used in cattle, swine and poultry at dose ranges of 22–66 mg per kg of body weight. The drug is rapidly absorbed when administered by the oral or parenteral route, maximum blood concentrations being reached in 1–5 hours. The major route of excretion in pigs and cattle is via the urine.

Chloramphenicol had previously been evaluated at the twelfth and thirty-second meetings of the Committee (Annex I, references 17 and 80). At the thirty-second meeting, the Committee was not able to establish an ADI because it was not possible to give an assurance that residues in foods of animal origin would be safe for human consumption, since it was concluded that human exposure to chloramphenicol could cause aplastic anaemia.

Toxicological data

Additional genotoxicity data together with new epidemiological data concerning the occurrence of aplastic anaemia in humans were available. In addition, the Committee re-evaluated previous data on chloramphenicol, which were summarized in the toxicological monograph published after the thirty-second meeting (Annex I, reference 81).

Information from a range of studies on chloramphenicol was considered, including data on pharmacokinetics, acute toxicity, carcinogenicity, tera-
toxicity and genotoxicity (including new studies). New epidemiological data were also evaluated.

Chloramphenicol was rapidly and extensively absorbed after oral administration to both laboratory animals and humans. It was distributed to all major organs and tissues. In contrast to oral absorption, the systemic uptake of the drug from ophthalmic application appeared to be poor. The major route of excretion in animals and humans is via the urine (up to 90% of the administered dose, of which 15% is excreted as parent compound and the rest in the form of metabolites). A number of metabolites are formed, the major one being the glucuronide.

Single intravenous doses of chloramphenicol were moderately toxic to mice, the median lethal dose (LD₅₀) being 1300–1800 mg per kg of body weight. No repeat-dose studies on mice were available to the Committee.

The Committee concluded that no adequate carcinogenicity studies were available, and an evaluation report was not presented (Annex 1, reference 104, section 2.3). The International Agency for Research on Cancer came to the same conclusion in 1975, 1987 and 1989 (5-7).

The original genotoxicity studies and the new data suggested that chloramphenicol and its metabolites were genotoxic both in a number of in vitro test systems and in an in vivo study on chromosomal aberrations in mice. The only negative study was a rat micronucleus test.

Rabbits given chloramphenicol orally at doses of 0, 500, 1000 or 2000 mg per kg of body weight per day for days 6-15, 6-9 or 8-11 of gestation showed high incidences of fetal deaths. There was no evidence of a teratogenic effect. The NOEL was 500 mg per kg of body weight per day. In a series of studies in the rat, embryolethality occurred even at the lowest dose tested (500 mg per kg of body weight per day). When mice were given oral doses of 500-2000 mg per kg of body weight per day, embryotoxicity was seen at all doses, but there was no evidence of a teratogenic effect.

No adequate reproduction studies were available and an evaluation report was not presented to the Committee (Annex 1, reference 104, section 2.3).

The original epidemiological evidence reviewed by the Committee suggested that treatment of humans with chloramphenicol was associated with the induction of blood dyscrasias, particularly aplastic anaemia. The Committee considered the new epidemiological data on aplastic anaemia, which showed that the total incidence was of the order of 1.5 cases per million people per year. Only about 15% of the total number of cases were associated with drug treatment, and chloramphenicol was not one of the main drugs implicated in such cases. These data gave an overall incidence of chloramphenicol-associated aplastic anaemia in humans of less than one case per 10 million per year. The Committee considered epidemiological data derived from the ophthalmic use of chloramphenicol, and concluded that the systemic exposure from this form of treatment
was not associated with the induction of aplastic anaemia. However, the Committee was unable to quantify the actual systemic exposure from ophthalmic use.

The Committee noted the extremely low overall incidence of aplastic anaemia and the lack of association between the ophthalmic use of chloramphenicol and this disorder. It concluded that human exposure to chloramphenicol residues in food of the same order as that resulting from systemic uptake after ophthalmic use would not cause any demonstrable alteration in the incidence of aplastic anaemia.

Toxicological considerations were of far greater concern than microbiological effects.

The Committee was unable to establish an ADI for chloramphenicol both because of the lack of the information needed to assess its carcinogenicity and effects on reproduction, and because the compound was genotoxic in a number of in vitro and in vivo test systems.

Residue data
The plasma kinetics of orally administered $^{14}$C-labelled chloramphenicol was studied in calves, pigs and poultry, and the results were comparable in all three species. The parent drug was the most abundant residue in poultry and calves and a major residue in pigs. Its concentration decreased at about the same rate as that of total residues in all three species.

The sponsors had submitted new information on metabolism in cattle (calves), pigs and poultry. They identified the specific metabolites after administration of a single oral dose of $[^{14}C]$chloramphenicol and estimated the residues in blood, plasma, muscle, liver, kidney and fat. The parent drug was present in all three species together with the following metabolites: chloramphenicol glucuronide, chloramphenicol base and hydroxyamphenicol.

There were numerous differences between the metabolic profiles in the three farm animal species. The profile for pigs was quite different from those for calves and poultry except in the case of muscle tissue. Furthermore, three of the known genotoxic and cytotoxic metabolites, nitrosochloramphenicol, dehydrochloramphenicol and dehydrochloramphenicol base, were not detected at the level of sensitivity of the assays in any species.

No radiolabelled chloramphenicol-depletion studies for residues in edible tissues of cattle or pigs over an extended period were available. It was therefore not possible to determine the concentration of total residues nor the percentage of parent drug and its metabolites in those residues during depletion. The data presented, however, did make it possible to identify and quantify the residues at the time intervals following a single oral dose when the concentration of radioactivity was at a maximum in the plasma (3 hours in pigs and 5 hours in calves).
Two radiolabelled chloramphenicol-depletion studies were available for poultry. In one study, depletion was followed over a 24-hour period after a single oral dose. In the other, birds were killed over a 17-day period after repeated oral administration of unlabelled drug followed by a final oral dose of radiolabelled chloramphenicol.

The radioactivity associated with the total residues and the metabolites was determined. The concentration of the metabolites was also determined by high-performance liquid chromatography (HPLC) with ultraviolet detection. In the repeat-dose study over a 17-day period it was not possible to measure the concentration of the total residues or the metabolites by means of radioactivity because the contribution of the unlabelled residues was unknown. The measurement of unlabelled residues as part of this study provided evidence of the persistence of chloramphenicol in skin at concentrations greater than 100 μg/kg, but no other metabolites could be found in this tissue at the 3-, 10- and 17-day sampling points.

The rates of depletion of the radioactivity in skin and muscle were comparable after a single administration of chloramphenicol; over 24 hours the depletion curves were more or less superimposed. After repeated administration of the drug to poultry over 96 hours, the decrease in radioactivity monitored over 17 days was slower in skin than in muscle, where the amounts recorded were at quantification limits. In this case total radioactivity in muscle was 57% of the level recorded in the skin 24 hours after administration of radioactive chloramphenicol. No metabolite could be found in muscle by HPLC with ultraviolet detection (less than 25 μg/kg) whereas there were very high concentrations (280–1180 μg/kg) of chloramphenicol residues in skin. This means that there might be unidentified residues in muscle and that skin accumulates mainly unmetabolized chloramphenicol.

No results were presented for bound chloramphenicol residues in any species.

Numerous analytical methods suitable for monitoring/controlling chloramphenicol residues at concentrations of 1 μg/kg or 1 μg/litre are available.

**Maximum Residue Limits**

The Committee was unable to assign MRLs for chloramphenicol primarily because no ADI was allocated. In addition, insufficient information was available to identify a suitable marker residue, particularly in cattle and pigs, for which radiodepletion studies were inadequate.

### 3.2.2 Flumequine

Flumequine had not previously been reviewed by the Committee. It is a first-generation quinolone with antimicrobial activity against Gram-negative organisms and is used for the treatment of infections in animals.
Toxicological data

Information from a range of studies on flumequine was available for assessment, including data on pharmacokinetics, acute toxicity, short-term and long-term toxicity, reproductive and developmental toxicity, and genotoxicity.

Studies with $^{14}$C-labelled flumequine in dogs and rats indicated that it is readily absorbed following oral administration. Most of the radioactivity in plasma appeared to be unchanged drug. The plasma half-life for flumequine was 5.25 hours in the rat. There were some differences between dogs and rats in the pattern of drug excretion. In dogs, 55–75% of the dose was excreted in the faeces, while in rats only 10–15% was excreted by this route. Less than 5% of the dose was present in the urine of dogs as unchanged drug and 13–15% as a conjugate of flumequine. In rats, 20–36% was eliminated in urine as unchanged drug and very little as a conjugate of flumequine.

Single oral doses of flumequine were slightly toxic ($LD_{50} = 1630–2210$ mg per kg of body weight) in rats, mice and rabbits.

The most common findings in rats dosed with 800 mg per kg of body weight per day by gastric tube for 14 days included bloating, cyanosis, dehydration, reduced weight gain and alopecia. In a 90-day study in rats, animals receiving doses of 0, 200, 400 or 800 mg per kg of body weight per day by gastric intubation showed a dose-dependent significant increase in relative liver weights. When guinea-pigs were repeatedly dosed with 300 or 500 mg per kg of body weight per day by gavage for 14 days, two of six animals died at the 300 mg dose. All animals receiving 500 mg per kg of body weight per day died after 6 days of treatment.

Repeated oral administration to dogs at 300 mg per kg of body weight per day for 21 days resulted in emesis, depression, ataxia and mild hyperactivity, but there were no deaths or consistent signs of toxicity at 200 mg per kg of body weight per day given in divided doses. In a 1-year study in dogs given total daily oral doses of up to 200 mg per kg of body weight per day, the most prominent effects were neurological signs. The NOEL was 50 mg per kg of body weight per day.

In an 18-month carcinogenicity study in mice, flumequine was administered in the feed at 0, 400 or 800 mg per kg of body weight per day. The only clinical signs were a slight depression in body weight, which occurred in the high-dose group from the sixth week to termination of the study. The incidence of benign and malignant liver tumours combined was dose-related, with incidences of 9%, 37% and 88% in the control, low-dose and high-dose males, respectively, and 0%, 0% and 13% in the control, low-dose and high-dose females, respectively. Dose-related toxic changes in the hepatocytes, which paralleled the liver tumour incidence, occurred in the low-dose males and in the high-dose males and females. The incidence of hepatotoxicity was statistically significant in all treated male groups and in the high-dose female group. A NOEL could not be determined in this study.
A further 18-month dietary study of flumequine in mice was conducted to investigate the relationship between duration of drug administration and the development of toxic and neoplastic liver lesions. Interim necropsies of males receiving 800 mg per kg of body weight per day revealed a time-dependent development of liver tumours following flumequine administration. At 3 months, 0/10 mice had liver tumours, but the same 10 mice all had evidence of toxic changes in the liver. The number of mice with liver tumours at 6, 9 and 12 months was 1/10, 3/10 and 9/10, respectively. Malignant tumours were observed in two of the animals killed at 12 months; the tumours observed in all other animals were benign.

In a 2-year carcinogenicity study in rats dosed with flumequine at 0, 200, 400 or 800 mg per kg of body weight per day, no carcinogenic effects were observed. Dose-related decreases in mean body weight and food consumption occurred. Spermatogenesis was absent in many animals in the mid- and high-dose male groups, and significant increases in the mean relative weight of the pituitary gland, liver, heart and brain were noted. A similar effect was observed in the liver, heart and brain of females in the high-dose group. At the high dose, the liver showed foci of swollen hepatocytes due to mild degenerative changes and, in a few cells, fatty changes were also seen. The NOEL was 200 mg per kg of body weight per day.

There was evidence of compound-related tumorigenic effects in the liver of mice. The Committee noted that the tumorigenic activity of flumequine was most pronounced in the liver of male mice, which are known to be sensitive to liver tumour induction. As the compound was negative in a range of mutagenicity studies, the mechanism of this tumorigenesis is unclear.

A study on the effect of flumequine on fertility and general reproductive performance was conducted in rats. In an unconventional protocol, groups of male and female rats were treated by gavage with doses of flumequine of 0, 100, 200, 400 or 800 mg per kg of body weight per day. Virtually all females in the highest-dose group died within 2 weeks of initiation of treatment. Because of the reduced body weight of pups at all doses, a NOEL could not be determined, but the Committee noted that there were no effects on fertility or on the reproductive performance of the dams.

In a teratology study, groups of mice were given doses of 0, 50, 100, 200 or 400 mg of flumequine per kg of body weight per day by gavage on days 6-15 of gestation. Incomplete ossification of sternebra and skull bones were noted in many of the fetuses in all groups, including the controls. The NOEL was 400 mg per kg of body weight per day in this study.

In another teratology study, groups of mice received doses of 0, 100, 200 or 400 mg of flumequine per kg of body weight per day by gastric tube
on days 2-15 of gestation. Increased frequency of cleft palate was considered indicative of fetotoxicity and compound-related in the mid- and high-dose groups. The NOEL was 100 mg per kg of body weight per day.

In a further teratology study, groups of rats were dosed orally with 0, 100, 200 or 400 mg of flumequine per kg of body weight per day on days 6-15 of gestation. There was a significant reduction in mean body weight at 400 mg per kg of body weight per day. In the mid- and high-dose groups, there was a significant reduction in mean fetal weights and incomplete ossification of sternebra, vertebrae and skull bones. No drug-related visceral or skeletal malformations were observed, and no embryotoxic effects were noted in this study. The NOEL was 100 mg per kg of body weight per day.

In a rabbit teratology study, animals were dosed by gavage with 0, 100, 200 or 400 mg of flumequine per kg of body weight per day on days 6-18 of gestation. No drug-related visceral or skeletal teratogenic changes or embryotoxic effects were noted in this study. The NOEL was 400 mg per kg of body weight per day.

The Committee recognized the association between quinolone exposure and arthropathy, and concluded that the data available to evaluate this hazard were not adequate for flumequine.

Genotoxicity studies in an in vitro bacterial and mammalian gene mutation assay and in an in vivo mammalian chromosome aberration assay were negative.

No data were available that would permit the Committee to evaluate the microbiological hazard of flumequine residues in food.

Residue data
Residue data were not provided for evaluation.

Appraisal
An ADI was not set for flumequine owing to the absence of the information outlined below.

Before reviewing flumequine again, the Committee would wish to see:

- Further data from mice from which NOELs for hepatotoxicity could be identified.
- Information on the tumorigenic mechanism of flumequine.
- Further data relating to the possible induction of arthropathy by the compound.
- Information on the microbiological safety of flumequine residues.
- Appropriate residue data.
3.2.3 Olaquindox

Olaquindox is an antimicrobial drug with a long history of use as a growth promoter in pigs. The commercial product is intended for use in starter and/or grower rations but not in finisher rations. It is usually administered in the feed at doses of 25-100 mg/kg and may be used in pigs up to 4 months of age.

Olaquindox had been previously evaluated at the thirty-sixth meeting of the Committee (Annex 1, reference 91). At that time, the Committee was unable to establish an ADI because of uncertainty regarding the mechanism involved in the slight increase in the incidence of adrenal cortical adenomas in male mice and the genotoxic potential of the drug, and it requested information on the toxic potential of the residues. In the meantime, the Committee concluded that residues resulting from the use of olaquindox in food-producing animals under conditions of good practice in the use of veterinary drugs were temporarily acceptable.

Toxicological data
In its thirty-sixth report, the Committee summarized a wide range of studies on biochemical aspects, short-term toxicity, long-term toxicity/carcinogenicity, reproductive toxicity and embryotoxicity/fetotoxicity, and special studies on genotoxicity and pharmacological effects.

The current residue studies confirm that olaquindox is extensively metabolized. Repeated oral administration to mice, rats and pigs produced a large number of metabolites at short withdrawal times. The metabolites detected varied, depending on the tissues and species investigated. All those found in the target species were also present in the tissues of rodents, and it was therefore possible to conclude that the general toxicity of all metabolites had been tested.

The Committee concluded that the toxicological concern associated with olaquindox residues when the drug is used in accordance with good practice in the use of veterinary drugs overshadowed any level of concern for microbiological effects.

The Committee also concluded that, because of the genotoxic potential of the parent compound and the absence of specific toxicity studies on the metabolites, it was still unable to allocate an ADI. However, it noted that the parent drug was absent in muscle at the proposed withdrawal time and that the toxicity of the metabolites could be partially evaluated on the basis of toxicity studies in experimental animals because the metabolites are similar to those in the target species. The Committee extended the temporary acceptance of residues resulting from the use of olaquindox in pigs in accordance with good practice in the use of veterinary drugs.

Residue data
In the Committee’s first review at the thirty-sixth meeting (Annex 1, reference 91), several conclusions were drawn from radiometric studies regarding metabolism and pharmacokinetics. The drug is rapidly absorbed
from the gastrointestinal tract, the major route of excretion being via the urine. There was no evidence of bound residues. Radiometric studies of duration less than 48 hours showed that the parent drug accounted for 70% of the total residues in urine. Five other metabolites were identified that involved side-chain oxidation and/or reduction of the N-oxide groups. The Committee further concluded, however, that there was insufficient information to enable it to complete its assessment of olaquindox. Before it could recommend MRLs, it therefore requested specific studies and data that would make it possible to characterize further the metabolites in tissues and to identify a marker residue suitable for regulatory purposes, together with an analytical method that could be used for such purposes (low µg/kg concentrations) at withdrawal times in excess of 48 hours. The Committee was aware at its thirty-sixth meeting that such residue and metabolite identification data and new analytical methods were being generated by the manufacturer. The studies requested were available for consideration at the present meeting of the Committee.

Four new total-residue-depletion studies in pigs were available in which radiolabelled drug was used. In three of the studies, a dose of 5 mg per kg of body weight was administered in gelatine capsules. In the fourth, an oral dose of 2.5 mg per kg of body weight was given, divided into two doses, of which the first was administered 12 hours before the second. Semilogarithmic plots showed linear depletion of total residues in muscle, liver and kidney. Olaquindox residues were proportional to the dose administered, and their concentration decreased in all the tissues measured at a constant rate; no bound residues appear to be present in tissue. At the recommended feed inclusion dose of 2.5 mg per kg of body weight per day and a 28-day withdrawal period, olaquindox residues in muscle are less than 5 µg/kg and less than 10 µg/kg in kidney.

In a radiolabelled olaquindox-depletion study using a single dose of 2 mg per kg of body weight and withdrawal times of 2 and 7 hours, parent drug and two mono-N-oxides could be identified in blood plasma. No olaquindox residues could be identified in plasma at 24 and 48 hours withdrawal. Organ tissues did not contain sufficient radioactivity 48 hours after dosing to enable olaquindox residues to be measured.

In a separate metabolism study at a dose of 5 mg per kg of body weight per day of 14C-labelled olaquindox for 7 days, the metabolites in liver and kidney were investigated. Of the total residue in liver 79% was recovered, and of that in kidney 93% was recovered when the pigs were killed 2 hours after the last dose. More than 80% of these extracted residues were identified as parent drug in which the bis-N-oxide group had been reduced. This compound was subsequently isolated from liver tissue in a feeding study with unlabelled olaquindox. A second metabolite was also isolated from liver tissue, in which it accounted for 13% of the total residues. Its structure showed it to be the carboxylic acid derivative of the bis-N-oxide-reduced parent compound.
In subsequent residue analysis following two long-term studies at doses of 25 and 100 mg/kg of unlabelled olaquindox in feed, the manufacturer identified the metabolite 3-methylquinoxaline-2-carboxylic acid (MQCA). Metabolites with N-oxide structures were not present in edible tissues of pigs at a 28-day withdrawal period. This structural element is known to be responsible for the bacterial mutagenicity of other quinoxaline derivatives. The manufacturer proposed MQCA as the marker compound for residues of olaquindox in muscle and liver tissue. Earlier radiolabel studies showed that liver had the highest residue concentration for up to 28 days after dosing. At longer withdrawal periods, the concentrations in muscle and liver become comparable in magnitude. In view of the analytical difficulties with liver tissue, muscle would be a suitable target tissue for residue analysis and control.

Residue studies at a dose of 25 mg/kg in feed indicate that depletion of residues in muscle tissue measured as MQCA is linear with time over the 8-20 day withdrawal period. In a 68-day study at 100 mg/kg of olaquindox in feed, steady-state concentrations of residues in pigs were reached 29 days after the start of medication.

Radiolabel studies indicate that the marker compound accounts for approximately 25% of the total residues. The identification of the remaining radioactivity in these studies was not possible. No studies were available in which the residues in liver or kidney tissue were measured as MQCA at the recommended dose of 50 mg/kg in medicated feed, or that indicated what proportion of the total residues was identified as MQCA in liver and kidney.

A new residue method is available in which the marker compound is quantified at 1 μg/kg in muscle tissue. In this method, gas chromatography/mass spectrometry is used to identify and quantify the methyl ester of MQCA by comparison with a [13C]MQCA internal standard. The response ratio of MQCA internal standard to MQCA is linear over the range 0.5–10 μg/kg. The method has been reported to be quantitative at concentrations of 0.5 μg/kg.

The Committee concluded that a residue concentration in muscle of 4 μg/kg, measured as MQCA, is consistent with the use of olaquindox in pigs in accordance with good practice in the use of veterinary drugs. This is based on the use of the recommended dose of 50 mg/kg of olaquindox in medicated feed in pigs up to approximately 16 weeks of age and a 28-day withdrawal period. Data on olaquindox residues in liver and kidney, measured as MQCA, at the recommended dose in pigs noted above were not available. In all previous cases the Committee has recommended MRLs in at least two tissues, so that the tissue with the highest residue can be used for national monitoring and the tissue of commerce can be monitored for trade purposes.

The Committee requires for review by 1996 the results of studies to determine residues in liver and kidney of pigs, MQCA being used as the marker residue.
3.2.4 Spectinomycin

Spectinomycin is an aminocyclitol antibiotic produced by *Streptomyces spectabilis*. It is used in human medicine for the treatment of uncomplicated gonorrhoea. In veterinary medicine, spectinomycin is used therapeutically for bacterial respiratory and enteric infections. It is administered to cattle, pigs and poultry as injectable solutions and orally as aqueous solutions or in feed. Spectinomycin had not been previously reviewed by the Committee.

**Toxicological data**

A range of studies on spectinomycin was submitted for assessment including data on pharmacokinetics and metabolism, as well as information from acute, short-term, reproductive, developmental and genotoxicity studies.

Toxicokinetic studies suggested poor absorption after oral dosing in rats, dogs, pigs and cattle, most of the dose being found in the faeces. Absorption is also poor in humans following oral administration. No data were available on biotransformation in animals, but limited information in humans suggested that the drug is not extensively metabolized.

Single oral doses of spectinomycin were of low toxicity to mice and rats (LD₅₀ = 3000-20000 mg per kg of body weight), although the drug appeared to be more toxic to dogs (LD₅₀ = 1000 mg per kg of body weight) and monkeys (LD₅₀ = 500 mg per kg of body weight).

No major toxicological effects were noted following repeated oral or parenteral administration to rats and dogs. The most common findings were related to changes in the consistency of the faeces in treated animals; the no-effect levels for these findings varied from 50 to 750 mg per kg of body weight per day.

Adequate carcinogenicity studies were not available. However, both *in vitro* and *in vivo* genotoxicity studies covering a variety of end-points were all negative, and spectinomycin is not structurally similar to known carcinogens. The Committee was therefore of the opinion that the drug did not present a carcinogenic risk and carcinogenicity studies were not deemed necessary.

A multigeneration reproduction study in rats showed no adverse effects on reproductive parameters up to the highest dose tested (400 mg per kg of body weight per day orally). Hepatocellular swelling and clumped basophilic material in the cytoplasm of hepatocytes were noted. This occurred only in some animals of the F₁₀ generation. The NOEL was 100 mg per kg of body weight per day.

Developmental toxicity was examined in mice, rats and rabbits. Spectinomycin was not teratogenic in rats after oral doses of up to 3000 mg per kg of body weight per day. There was no evidence of teratogenic effects after intraperitoneal or subcutaneous administration in this
species. It was not teratogenic in mice after intraperitoneal doses of up to 1600 mg per kg of body weight per day. The study in rabbits revealed no teratogenic effects after subcutaneous or intramuscular doses of up to 300 mg per kg of body weight per day.

There was no evidence of ototoxicity in cats after intramuscular doses of up to 120 mg per kg of body weight per day for periods of 75-90 days. The Committee concluded that no major toxicological effects were associated with spectinomycin in humans or animals.

Microbiological data
The potential for adverse effects on the human gut flora was considered. In vitro data on the minimum inhibitory concentration (MIC), covering a wide range of animal and human pathogens and commensals, were submitted for assessment.

The Committee examined MIC data for a number of bacterial species representative of the anaerobic microbial flora in the human gastrointestinal tract, including Bacteroides, Peptostreptococcus, Fusobacterium, Eubacterium and Clostridium spp. Many had MIC₅₀ values greater than 50 μg/ml. Bifidobacterium spp. were more sensitive, with MIC values for spectinomycin in the range 2-32 μg/ml. The modal MIC¹ was 16 μg/ml with an inoculum density of 10⁶ bacteria/ml and 8 μg/ml with a density of 10⁵ bacteria/ml. The Committee used the value of 16 μg/ml (assumed to be equivalent to 16 μg/g) in its calculations.

In calculating the ADI, the Committee used the formula developed at the thirty-eighth meeting of the Committee (Annex 1, reference 97):

\[
\text{Upper limit of ADI (μg/kg of body weight)} = \frac{\text{Concentration without effect on human gut flora (μg/ml) \times daily faecal bolus (g)}}{\text{Fraction of oral dose bioavailable} \times \text{Safety factor} \times \text{Weight of human (80 kg)}}
\]

\[
= \frac{16 \times 150}{1 \times 1 \times 60}
\]

\[
= 40 \text{ μg per kg of body weight}
\]

It established safety factors as follows:

- Factors to account for the range of MICs needed to allow for sensitive bacteria, anaerobic environment, bacterial density and pH: the Committee concluded that sufficient experimental data had been provided and that no specific factors were required to adjust the modal MIC of 16 μg/ml to a "microbiological no-effect level".
- Availability: the Committee noted that absorption of spectinomycin from the gastrointestinal tract was poor. It therefore took a conservative

¹ For the purpose of this evaluation, the modal MIC means the most frequently observed MIC in a frequency distribution of MICs for strains of the relevant species tested.
approach, assuming that the availability of ingested spectinomycin to organisms in the gastrointestinal tract was 100%.

Variability among exposed individuals: a substantial amount of MIC data covering a variety of organisms was available. In addition, recently published data suggested that variability among populations was low. A safety factor of 1 was therefore adopted by the Committee.

Taking all of these factors into account, the Committee established an ADI of 0–40 µg per kg of body weight for spectinomycin. In view of the extensive range of organisms examined, the provision of data on the effects of pH, inoculum density and resistance to spectinomycin, and taking into account the discussions during the meeting on the microbiological safety of residues (section 2.2), the Committee considered that, in this instance, a final ADI was appropriate.

Residue data
Spectinomycin is poorly absorbed from the gastrointestinal tract of pigs and cattle; the oral bioavailability factor is therefore about 10%. After intramuscular injection, in contrast, spectinomycin is well absorbed, and the bioavailability factor is nearly 100%. It is eliminated mainly unchanged in the urine. Repeated administrations do not result in drug accumulation. Following oral administration in pigs, spectinomycin concentrations in plasma were below 30 µg/kg at all times tested; after intramuscular injection, however, concentrations were above 30 µg/kg for up to 12 hours after treatment. In cattle, the results obtained following single doses of 10 mg per kg of body weight by intramuscular or intravenous administration were similar to those obtained after multiple dosing. Plasma concentrations were greater than 0.1 mg/kg for up to 12 hours after treatment. The half-life in bovine plasma was around 2 hours.

Spectinomycin residue-depletion studies were performed in pigs, cattle and chickens; the residues were measured by microbiological or more sensitive HPLC methods. Dose levels of spectinomycin by intramuscular injection in several studies ranged from 10 to 20 mg per kg of body weight. Depletion studies were also conducted in pigs and cattle with tritiated spectinomycin. In all studies, the kidney had the highest and most persistent residue concentrations and was therefore chosen as the target tissue. Concentrations of residues were lower in liver, the lowest levels being found in muscle and fat. After multiple intramuscular doses of spectinomycin (10 mg per kg of body weight) in calves, the highest residue concentrations (15.5 mg/kg) were observed in the kidneys at 8 hours withdrawal time, declining to 1.4 mg/kg at 7 days. Residues in liver depleted from 3.3 mg/kg at 8 hours to 0.6 mg/kg at 7 days. Residues in muscle at 7 days after the last treatment were less than 0.1 µg/kg.

In pigs after 7 days of intramuscular treatment, the highest spectinomycin residue concentrations (15.1 mg/kg) were found in kidneys 12 hours after treatment. At 96 hours withdrawal time, the residues declined to 0.8 mg/kg.
Milk from dairy cows dosed intramuscularly with 20 mg/kg of body weight twice daily for 3 consecutive days showed spectinomycin concentrations below 0.2 µg/ml at the fifth milking after the last dose.

Spectinomycin can be assayed in plasma, urine, milk and tissues by means of a microbiological cylinder plate assay with a detection limit of 1.0 µg/g. HPLC methods for tissues in all species and for milk are available with a detection limit of 0.1 µg/g.

**Maximum Residue Limits**
Based on the ADI of 0-40 µg per kg of body weight established by the Committee, the following temporary MRLs were recommended for pigs, cattle and chickens: kidney 5000 µg/kg, liver 2000 µg/kg, muscle 300 µg/kg and fat 500 µg/kg as parent drug. The temporary MRL recommended for milk is 200 µg/l.

These MRLs were designated as temporary because many of the residue-depletion data reviewed by the Committee were either from interim progress reports or from pilot studies. The Committee was also aware that additional metabolism studies were being conducted to confirm that the microbiologically active portion of the residues in edible tissues is predominantly spectinomycin. The results of these studies are required for review by 1996.

From these values for the MRLs, the theoretical maximum daily intake of spectinomycin residues is 865 µg/day. This is considerably less than the maximum ADI of 2.4 mg for a 60-kg person.

3.2.5 **Sulfadimidine**
Sulfadimidine is a sulfonamide used to treat a variety of bacterial diseases in humans and other species and to promote growth in food-producing animals. It had previously been reviewed at the thirty-fourth meeting of the Committee (Annex 1, reference 85), when a temporary ADI of 0-4 µg per kg of body weight was established based on a NOEL of 2.2 mg per kg of body weight per day for thyroid follicular cell hyperplasia in the rat and a safety factor of 500. At that time, the Committee was aware of additional studies in progress on the mechanism of action of sulfadimidine on the thyroid gland, and requested that the results of those studies should be submitted by 1991. At the thirty-eighth meeting (Annex 1, reference 97), the final reports were not available and the Committee extended the temporary ADI.

At its present meeting, these studies and additional information regarding genotoxicity, embryotoxicity and teratogenicity were reviewed.

**Toxicological data**
In a teratogenicity study on rats dosed orally with 0, 540, 680 or 860 mg per kg of body weight per day, the incidence of cleft palate and minor
visceral malformations was higher at the two highest doses, giving a NOEL of 540 mg per kg of body weight per day. In a similar study in rabbits at dose levels up to 1800 mg per kg of body weight per day, no malformations were observed, but there were dose-related increased incidences of resorptions and fetal deaths per litter. The NOEL for embryotoxicity was 1200 mg per kg of body weight per day.

A range of in vitro and in vivo genotoxicity tests were generally negative. A positive result was obtained with a sister chromatid exchange assay in the absence of metabolic activation. The Committee noted that the protocol did not meet current standards.

In several short-term toxicity studies with rats given sulfadimidine in the diet up to a dose of 600 mg per kg of body weight per day, an increase in thyroid weight, decreases in the circulating concentration of the thyroid hormones tri-iodothyronine (T3) and thyroxine (T4), and an increase in thyroid-stimulating hormone (TSH) were observed. These changes were accompanied by hypertrophy and hyperplasia of thyroid follicular cells. The overall NOEL in these studies was 5 mg per kg of body weight per day. In pigs given sulfadimidine in the diet for 4 weeks at doses of 0, 5, 10, 20 or 40 mg per kg of body weight per day, similar effects were observed, the NOEL being 5 mg per kg of body weight per day. In monkeys given sulfadimidine orally at 0, 30, 100 or 300 mg per kg of body weight per day, no effects on the thyroid gland were observed.

The Committee noted that the studies currently available dealt with relevant and sensitive endpoints with respect to the toxic effects of sulfadimidine on the thyroid gland. It was concluded that the tumors seen at high dose levels of sulfadimidine are due to the enhanced hormonal stimulation of the thyroid gland caused by the elevated TSH levels produced by the drug and not by its direct action.

The Committee also considered the formation of the reactive diazonium intermediate produced in the gastrointestinal tract by bacterial action. It recognized that the diazonium ion binds covalently to intestinal contents, and therefore concluded that it was of no further toxicological concern.

In the light of all the available information, including the studies evaluated at the thirty-fourth meeting (Annex 1, reference 85), the Committee established an ADI of 0-50 μg per kg of body weight based on an overall NOEL of 5 mg per kg of body weight per day observed in rats and pigs for changes in thyroid morphology, and a safety factor of 100.

Effects of sulfadimidine on human health

Although it was recognized that primates (including humans) are less susceptible than rats and pigs to the antithyroid effect of sulfonamides, the Committee noted the possibility that, in the case of sensitization to sulfonamides, hypersensitivity reactions might occur as a result of the ingestion of sulfonamide residues in food of animal origin.
Residue data
No new information was available on sulfadimidine residues for the Committee to consider. The report of and residues monograph produced at the thirty-fourth meeting of the Committee (Annex 1, references 85 and 87) summarize the results of studies on residues of sulfadimidine in pigs, cattle, sheep and poultry.

Maximum Residue Limits
The Committee recognized that information on hypersensitivity reactions resulting from the ingestion of tissues containing sulfonamides (in general) would be extremely difficult, if not impossible, to obtain. In line with the previous evaluation (Annex 1, reference 85), the Committee therefore recommended that the MRLs should be set as low as is practicable in accordance with good practice in the use of veterinary drugs. In doing so, it recognized that these concentrations would then be below the levels considered significant from the point of view of microbiological concern. On this basis, the Committee concluded that the MRLs for sulfadimidine should remain as recommended at its thirty-fourth meeting since methods are routinely available for national residue monitoring. The values recommended for cattle, sheep, pigs and poultry are 100 μg/kg as sulfadimidine in muscle, liver, kidney and fat. The recommended MRL for milk as sulfadimidine is 25 μg/l. For these MRLs, the theoretical maximum daily intake of sulfadimidine residues is 87.5 μg/day, which is considerably less than the maximum ADI of 3 mg for a 60-kg person.

The residue data available for eggs indicate that compliance with current practices in egg production would result in very high concentrations of sulfadimidine in eggs. For this reason, the Committee considered that sulfadimidine should not be used in laying hens, and did not recommend an MRL for eggs.

3.3 Antiprotozoal agent
3.3.1 Ronidazole
Ronidazole had previously been evaluated at the thirty-fourth meeting of the Committee (Annex 1, reference 85), when a temporary ADI of 0-0.025 mg per kg of body weight was established. Additional data were required for consideration by the Committee.

New data were not made available to the Committee at the present meeting, and the temporary ADI was therefore not extended.

3.4 Glucocorticosteroid
3.4.1 Dexamethasone
Dexamethasone had not previously been evaluated by the Committee. It is a potent synthetic analogue of hydrocortisone that has a long history
of use in veterinary medicine for the treatment of a range of metabolic
diseases and inflammatory disorders in both companion and farm
animals. Animal diseases for which dexamethasone is an effective
treatment include inflammation, acetonaeemia, non-specific skin disease,
shock and stress. Its use in animals is primarily therapeutic, and it is also
used in human medicine for the treatment of a wide range of diseases.
This wide range of therapeutic uses reflects the broad spectrum of
pharmacological actions of the corticosteroid hormones, which exert
effects on several important biochemical pathways and cellular transport
mechanisms, including cellular sodium transport, glycogen synthesis and
anti-inflammatory responses.

Toxicological data
Information from studies on dexamethasone, including data on kinetics,
metabolism, acute and short-term toxicity, developmental toxicity and
genotoxicity, was available for assessment.

Toxicokinetic studies revealed rapid absorption after intramuscular
administration to dogs and rats, peak plasma levels being found after
30 minutes and 6 hours, respectively. Dexamethasone is rapidly excreted
in urine and faeces, and dexamethasone esters are rapidly hydrolysed
in serum. Biotransformation is similar in rats and humans, and involves
mainly hydroxylation to 6-hydroxy- and 20-dihydrodexamethasone.
However, there was also evidence that, at high (therapeutic) doses in
humans, dexamethasone is metabolized by another route involving
epoxidation.

After repeated oral administration of dexamethasone to rats and dogs in
short-term toxicity studies, the main target organs were the thymus and
the adrenal gland. Corticosteroid concentrations in plasma and hepatic
glycogen were reduced, whereas serum lipid levels were increased. In
rats dosed orally with 0.3, 1, 3, 10, 30 or 100 μg of dexamethasone per kg
of body weight per day for 90 days, thymus involution, morphological
changes in the adrenal gland, and a decrease in corticosterone and white
blood cell counts were observed in male and female rats at doses above
10 μg per kg of body weight per day. Because of the decrease in the white
blood cell count in female rats at 3 μg per kg body weight per day, this
dose was considered to be a marginal effect level. In a study on rats orally
dosed with 0.5, 1, 1.5, 2 or 4 μg of dexamethasone per kg of body weight
per day for 7 days, the corticosterone concentration was reduced in the
highest-dose group, and the activity of tyrosine aminotransferase in the
liver was increased in a dose-related manner at 2 and 4 μg per kg of body
weight per day. The NOEL in this study was 1.5 μg per kg of body weight
per day.

No reproduction studies with dexamethasone were available, but an
increase in pre- and post-implantation loss and a reduction in fetal weight
were observed in teratogenicity studies in mice, rats and rabbits receiving
dexamethasone by injection. In these studies, malformations such as
hydrops fetalis, cleft palate, exencephaly and encephalocele were observed at maternally toxic dose levels.

In oral teratogenicity studies with rats at dose levels ranging from 10 to 1250 µg per kg of body weight per day, maternal toxicity was found at 50 µg per kg of body weight and above. At doses at and above 1000 µg per kg of body weight per day, dexamethasone caused structural malformations (hydrops fetalis, cleft palate). Thymus involution and a decrease in body weight were observed in fetuses, resulting in an overall NOEL for embryotoxicity in rats of 10 µg per kg of body weight per day.

Although long-term toxicity/carcinogenicity data were not available, the Committee was not concerned about the carcinogenic potential of dexamethasone, in view of its long history of use in human medicine and the negative results obtained in in vitro gene mutation assays with bacteria and mammalian cells and in an in vivo micronucleus test with mice.

Using a safety factor of 100, the Committee established an ADI of 0–0.015 µg per kg of body weight for dexamethasone based on a NOEL of 1.5 µg per kg of body weight per day for the induction of tyrosine aminotransferase activity in rat liver. Because of the careful selection of the dose levels in this study, the Committee did not round up this figure.

Residue data
Dexamethasone esters are very efficiently and rapidly hydrolysed both in in vitro systems and in vivo by esterases. The relative dexamethasone residue concentrations in edible tissues following the administration of ester formulations therefore depends on the bioavailability of the ester from the injection site. However, the rate of absorption of the ester from the injection site differs for different ester formulations, and this rate determines the availability of dexamethasone.

The concentration of dexamethasone at the injection site at a particular time after injection will thus depend on the dexamethasone ester used, and will significantly exceed levels in all other edible tissues at the same time until it is totally absorbed.

Recent findings that tritiated dexamethasone is subject to tritium exchange in vivo render any residue-depletion data based on studies on radiolabelled drug conducted before 1992 open to challenge. Residue-depletion data for dexamethasone and its esters were therefore restricted. Recent data have been supplied by the manufacturer for the depletion of various esters in cattle and pig tissues. The results indicate that different ester preparations lead to significantly different dexamethasone-depletion rates.

Initial results suggested that in cattle and pigs:

- dexamethasone residues are quickly eliminated from muscle and cows' milk;
- residues do not occur in fat in the free form (no studies on the potential retention of dexamethasone esters in fat have been carried out);
- depletion of dexamethasone residues is slowest from liver, which is therefore the target tissue of choice.

Except for conjugates of dexamethasone itself, the major metabolic excretion pathway in all species studied commences with 6-hydroxylation of the steroid ring. This, together with minor metabolic pathways, results in a large decrease in corticosteroid activity. The low NOEL which has been established is based on the pharmacological activity of the free drug, since oxidized metabolites are inactive. The parent drug, dexamethasone, was therefore proposed as the marker residue.

A method for dexamethasone determination by HPLC/mass spectrometry is available with a lower quantification limit of 2.5 µg/kg for liver, 0.5 µg/kg for kidney and muscle, and 0.25 µg/kg for milk. This method is not available for peer review and is technically sophisticated. Routine immunoassay methods are available, but positive results require support by a confirmatory procedure.

**Maximum Residue Limits**

Based on the ADI of 0–0.015 µg per kg of body weight for parent drug established by the Committee, the acceptable daily intake of parent drug is 0.9 µg/day for a person weighing 60 kg.

The MRLs that satisfy the ADI criteria in cattle and pigs are 2.5 µg/kg for liver, 0.5 µg/kg for muscle and kidney, and 0.3 µg/l for cows’ milk. There was insufficient information to establish an MRL for fat. The maximum theoretical daily intake, based on the recommended MRLs and ingestion of 300 g of meat (as muscle), 100 g of liver, 50 g of kidney and 1.5 l of milk, is 0.875 µg. No allowance is made for dexamethasone residues at the injection site in this calculation.

The analytical method provided has quantification limits at the same concentrations as the required MRLs in all tissues.

The Committee proposed that the above-mentioned MRLs should be temporary until such time as it is satisfied that suitable methods for use in regulatory monitoring in all tissues at these MRLs are available. This information should be provided for consideration by 1996.

3.5 **Trypanocide**

3.5.1 **Diminazene**

Diminazene is a veterinary drug with a long history of use for the treatment of trypanosomiasis and babesiosis. It had previously been evaluated at the thirty-fourth meeting of the Committee (Annex 1, reference 85). At that time, the Committee was unable to establish an ADI and suggested that additional genotoxicity and teratogenicity studies should be performed before the compound was brought to the Committee for re-evaluation.
Toxicological data

Diminazene was negative in the micronucleus test and in bacterial and mammalian genotoxicity assays. These data, together with those from the subchronic toxicology studies, which showed no lesions predictive of a carcinogenic response, suggest that the carcinogenic potential of the compound is not a cause for concern.

The embryotoxicity of diminazene was examined in female Wistar rats dosed by gavage with 0, 200, 400 or 800 mg per kg of body weight per day on days 7–16 after mating. A dose of 800 mg per kg of body weight per day was maternotoxic, causing a decrease in food consumption, increased spleen weight and death. This dose also caused retarded fetal development. The NOEL for this study was 400 mg per kg of body weight per day. In another embryotoxicity study, diminazene was administered to rats by gavage on days 8–15 of pregnancy at dose levels of 0, 100, 250, 500 and 1000 mg per kg of body weight per day. The dose of 1000 mg per kg body weight per day produced maternotoxicity, a significant increase in the number of fetal resorptions, and a decrease in fetal body weights. The NOEL for this study was 500 mg per kg of body weight per day. No fetal malformations were detected in either study.

The previous monograph on diminazene (Annex 1, reference 86) summarized the toxicity studies in rats, dogs and various food-producing animals. At low doses, intramuscular administration of diminazene produced central nervous system lesions in several species including cows, donkeys, camels and dogs. In a study in which dogs were fed diminazene at 20 and 60 mg per kg of body weight per day for 9 months, the higher dose caused brain damage, testicular atrophy and death. Rats dosed by gavage at 63 and 160 mg per kg of body weight per day for 3 months showed no signs of toxicity. This indicates that the rat is not a sensitive model for evaluating the toxicity of diminazene, including its effects on reproduction. The NOEL in the study in dogs was 20 mg per kg of body weight per day.

In keeping with the general principles for the evaluation of veterinary drugs with a long history of use laid down at the fortieth meeting of the Committee (Annex 1, reference 104), an ADI of 0–100 µg per kg of body weight was established for diminazene based on the NOEL of 20 mg per kg of body weight per day in the 9-month study in dogs and a safety factor of 200. The Committee chose this safety factor to compensate for the inadequacies in the design of the study.

Residue data

The Committee received new information on methods of analysis for diminazene aceturate in bovine milk, muscle, liver and kidney. These were used to measure the residues in two studies in which either calves or lactating cows were given a single intramuscular injection of the drug at the recommended dose. No residues were detected in milk at any milking after dosing. The calves were sampled at 21, 28 and 35 days after dosing,
and the residues of parent drug were measured in muscle, liver, kidney and at the injection site. Over a period of 21–35 days after the injection of the drug, the half-life of the parent drug at the injection site in the liver and kidney was 6–8 days. It was not possible to estimate the half-life in muscle because the concentrations of the residues were too close to the quantification limits of the analytical method.

**Maximum Residue Limits**

Based on the ADI of 0–100 µg per kg of body weight for parent drug established by the Committee, the permitted daily intake of parent drug and/or its equivalents is 6000 µg for a 60-kg person.

From a comparison of the data for total residues at 20 days with the data for residues of diminazene at 21 days, parent drug accounts for approximately 22% of the total residues in kidney, about 27% in liver and 100% in muscle.

The Committee recommended MRLs for cattle of 12000 µg/kg for liver, 6000 µg/kg for kidney, 500 µg/kg for muscle and 150 µg/l in milk, expressed as the parent drug. The recommended MRL for milk was based on the quantification limit of the analytical method (as determined by a 10:1 signal-to-noise ratio for the method). If these values are used for the MRLs and it is assumed that unidentified drug residues in liver and kidney are equivalent to diminazene, the theoretical maximum daily intake of residue of parent drug and its equivalents is approximately 6200 µg, made up of 1875 µg of identifiable diminazene and approximately 4300 µg of unidentified diminazene equivalents (Table 2).

The method used to measure the residues was HPLC with ultraviolet detection, which has quantification limits of 150 µg/l for milk and 300 µg/kg for tissues.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Theoretical maximum daily intake of diminazene residues*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>MRL (µg/kg)</td>
</tr>
<tr>
<td>Muscle</td>
<td>500</td>
</tr>
<tr>
<td>Fat</td>
<td>—</td>
</tr>
<tr>
<td>Liver</td>
<td>12000</td>
</tr>
<tr>
<td>Kidney</td>
<td>6000</td>
</tr>
<tr>
<td>Milk</td>
<td>150</td>
</tr>
<tr>
<td>Total</td>
<td>1875</td>
</tr>
</tbody>
</table>

* Based on a daily intake of 0.5 kg of meat made up of 0.3 kg of muscle, 0.05 kg of fat, 0.1 kg of liver and 0.05 kg of kidney, and 1.5 l of milk.

b Although no MRL was recommended for fat, it can be assumed that the residue in fat is zero.
4. **Recommendations**

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

2. In view of the large number of veterinary drugs requiring evaluation, meetings of the Joint FAO/WHO Expert Committee on Food Additives should be held annually for this purpose.

**Acknowledgement**

The Expert Committee wished to acknowledge the valuable contribution made to its work by Dr Y. Yamada, Food Standards Officer, Joint FAO/WHO Food Standards Programme, Food Policy and Nutrition Division, FAO, Rome, Italy.

**References**


Annex 1

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


4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Expert Committee). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, vol. II. Food colours. Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).


24. **Specifications for the identity and purity of some extraction solvents and certain other substances**. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/FOOD Add/70.40.


55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives).* FAO Food and Nutrition Paper, No. 17, 1980.


58. *Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives).* FAO Food and Nutrition Paper, No. 19, 1981.


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 agent</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Levamisole | 0–6 μg/kg of body weight | Muscle, kidney and fat (cattle, sheep, pigs and poultry): 10 μg/kg<sup>a</sup>  
Liver (cattle, sheep, pigs and poultry): 100 μg/kg<sup>a</sup>  
Milk: previous temporary MRL withdrawn<sup>b</sup>  
Eggs: no MRL allocated<sup>c</sup> |
| **Antimicrobial agents** | | |
| Chloramphenicol | Not allocated<sup>d</sup> | No MRLs allocated<sup>d</sup> |
| Flumequine | Not allocated<sup>d</sup> | Not evaluated<sup>d</sup> |
| Olaquindox | Limited acceptance of residues<sup>e</sup> | Insufficient information available to establish MRLs in two tissues<sup>f</sup> |
| Spectinomycin | 0–40 μg/kg of body weight | Muscle (cattle, pigs and chickens): 300 μg/kg<sup>h</sup>  
Liver (cattle, pigs and chickens): 2000 μg/kg<sup>h</sup>  
Kidney (cattle, pigs and chickens): 5000 μg/kg<sup>h</sup>  
Fat (cattle, pigs and chickens): 500 μg/kg<sup>i</sup>  
Milk (cattle): 200 μg/l<sup>j</sup> |
| Sulfadimidine | 0–50 μg/kg of body weight | Muscle, liver, kidney and fat (cattle, sheep, pigs and poultry): 100 μg/kg<sup>a</sup>  
Milk (cattle): 25 μg/l<sup>a</sup>  
Eggs: no MRL allocated<sup>c</sup> |
| **Antiprotozoal agent** | | |
| Ronidazole | Temporary ADI not extended | Not evaluated<sup>g</sup> |
| **Glucocorticosteroid** | | |
| Dexamethasone | 0–0.015 μg/kg of body weight | Muscle and kidney (cattle and pigs): 0.5 μg/kg<sup>l</sup>  
Liver (cattle and pigs): 2.5 μg/kg<sup>l</sup>  
Fat: no MRL allocated<sup>m</sup>  
Milk (cattle): 0.3 μg/l<sup>n</sup> |
<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>Trypanocide</td>
<td></td>
<td>Muscle (cattle): 500 µg/kg&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diminazone</td>
<td>0–100 µg/kg of body weight</td>
<td>Liver (cattle): 12,000 µg/kg&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney (cattle): 6,000 µg/kg&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat: no MRL allocated&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk (cattle): 150 µg/kg&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Notes to Annex 2

<sup>a</sup> Expressed as parent drug.
<br>
<sup>b</sup> No new data were submitted by the sponsors to support the re-evaluation of levamisole residues in milk. The Committee therefore withdrew the temporary MRL for milk allocated at its thirty-sixth meeting. The sponsors recommend that levamisole should not be used in lactating cows.
<br>
<sup>c</sup> The Committee considered that levamisole should not be used in laying hens.
<br>
<sup>d</sup> An ADI was not established because the information necessary to assess the carcinogenicity and effects on reproduction of chloramphenicol was lacking and because the compound was positive in a number of in vitro and in vivo genotoxicity test systems.
<br>
<sup>e</sup> MRLs were not allocated primarily because an ADI was not established. In addition, sufficient information was not available for the Committee to establish a suitable marker residue, particularly for cattle and pigs, in which an inadequate number of radiorepletion studies were available.
<br>
<sup>f</sup> An ADI was not established because of the lack of information: (a) necessary to identify NOELs for hepatotoxicity; (b) on the tumorigenic mechanism; (c) on the possible induction of arthropathy; and (d) on the microbiological safety of residues.
<br>
<sup>g</sup> No data were available from which the residues could be assessed.
<br>
<sup>h</sup> The information available was insufficient to establish an ADI. The Committee concluded that residues resulting from the use of olaquindox in pigs in accordance with good practice in the use of veterinary drugs were temporarily acceptable, pending submission of the results of the requested studies on residues.
<br>
<sup>i</sup> The Committee concluded that a residue level in muscle of 4 µg/kg, measured as 3-methylquinoloxaline-2-carboxylic acid (MOCA), is consistent with the use of olaquindox in pigs in accordance with good practice in the use of veterinary drugs. However, the information available was not sufficient to enable an MRL, in a second tissue to be recommended. The Committee requires for review by 1996 the results of studies to determine residues in liver and kidney of pigs, MOCA being used as the marker residue.
<br>
<sup>j</sup> Temporary. Final reports on metabolism and residue studies in food-producing animals are required for review by 1996.
<br>
<sup>k</sup> The Committee considered that sulfadimidine should not be used in laying hens.
<br>
<sup>l</sup> Temporary. Suitable methods for use in regulatory monitoring at these MRLs are required for review by 1996.
<br>
<sup>m</sup> According to the sponsors no residues occurred in fat. However, it appeared to the Committee that the sponsors had not investigated the possibility of re-esterification and deposition of dexamethasone esters in fat.
<br>
<sup>n</sup> For the purpose of estimating the theoretical maximum daily intake of diminazene residues, it can be assumed that the residue in fat is zero.
<br>
<sup>o</sup> Quantification limit of the analytical method.
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Report of a WHO Study Group (29 pages) 6.–

848 (1994) WHO Expert Committee on Biological Standardization
Forty-fourth report (94 pages) 14.–

849 (1995) Control of foodborne trematode infections
Report of a WHO Study Group (165 pages) 26.–

Sixth report of the WHO Expert Committee (144 pages) 21.–