EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

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

World Health Organization
Geneva 1996
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Joint FAO/WHO Expert Committee on Food Additives
Geneva, 6-15 June 1995

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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. 41/8, 1996.

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**INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY**

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

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

A meeting of the Joint FAO/WHO Expert Committee on Food Additives was held at WHO headquarters, Geneva, from 6 to 15 June 1995. The meeting was opened by Dr M.J.G. Mercier, Director of the International Programme on Chemical Safety, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization.

Dr Mercier noted the problems that have been encountered in evaluating residues of antimicrobial agents in food, which stem primarily from the fact that fully validated tests for measuring the antimicrobial effects of such residues are not available. It is important that further progress in test methodology is made so that potential microbiological hazards can be assessed in a credible and consistent manner. Dr Mercier also brought to the Committee's attention the report of a Joint FAO/WHO Expert Consultation on the Application of Risk Analysis to Food Standards Issues (1), held in Geneva in March 1995. The Consultation considered risk analysis in the Codex Alimentarius system, the role played by the expert committees, including the Joint FAO/WHO Expert Committee on Food Additives, and uncertainty associated with the risk assessment process. It confirmed the importance of the role of the Committee in the risk assessment of residues of veterinary drugs in food, and addressed many of the issues outlined in section 2.1 of the report of the forty-second meeting of the Committee (Annex 1, reference 110).

Seven previous meetings of the Committee had been held to consider veterinary drug residues in food (Annex 1, references 80, 85, 91, 97, 104, 110 and 113) in response to the recommendations of the Joint FAO/WHO Expert Consultation held in 1984 (2). The present meeting\(^1\) was convened in response to the recommendation made at the forty-third meeting of the Committee that meetings on this subject should be held annually (Annex 1, reference 113). The Committee's purpose was to provide guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on public health issues pertaining to residues of veterinary drugs in foods of animal origin. The specific tasks before the Committee were:

- to elaborate further principles for evaluating the safety of residues of veterinary drugs in food and for establishing Acceptable Daily 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); and
- to evaluate the safety of residues of certain veterinary drugs (see section 3 and Annexes 2 and 3).

\(^1\) 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, 1960), there have been 44 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
2. **General considerations**

2.1 **Modification of the agenda**

Triclabendazole was removed from the agenda because no new data were submitted. Levamisole had been placed on the agenda for the assessment of residues in milk. However, the Committee considered that it was not necessary to review this drug, because the previous temporary MRL for milk had been withdrawn at the forty-second meeting (Annex 1, reference 110).

2.2 **Neurotoxicity of avermectins and milbemycins**

At its present meeting, the Committee considered three anthelmintics belonging to the avermectin and milbemycin classes. During its discussions, the Committee noted that a subpopulation of CF-1 mice had been shown to be more sensitive than CD-1 mice to the neurotoxic effects of ivermectin and abamectin (3). Since the Committee could not exclude the possibility that similarly sensitive groups exist in human populations, it discussed in some detail the implications of the observed neurotoxic effects in CF-1 mice for its ADI recommendations for avermectins and milbemycins.

In a short-term study, comparisons were made of the sensitivity of CF-1 and CD-1 mice to the neurotoxic effects of abamectin, to determine whether deficiency of P-glycoprotein (a component of the plasma membrane which controls the rate of passage of xenobiotics across membranes) could explain the high sensitivity of some CF-1 mice. Signs of severe neurotoxicity were seen in some CF-1 mice but not in CD-1 animals. Immunohistochemistry of the cerebellum, cerebral cortex and jejunum showed that the abamectin-sensitive CF-1 mice had very low levels of P-glycoprotein compared with both CD-1 mice and CF-1 mice not sensitive to the effects of abamectin (4). Mice which, as a result of genetic modification, are unable to express the P-glycoprotein gene have also been shown to be highly sensitive to the neurotoxic effects of ivermectin (5). Hence, there appears to be a strong correlation between levels of P-glycoprotein and the neurotoxic effects of abamectin and ivermectin.

A subpopulation of CF-1 mice may therefore be more sensitive to the effects of avermectins and milbemycins because they have low levels of P-glycoprotein. The Committee also noted that some collie dogs and Murray Grey cattle are also more sensitive to the neurotoxic effects of some avermectins (6, 7). Despite the widespread use of this class of compounds in humans without apparent adverse effects, the Committee could not exclude the possibility that some sensitive human populations may exist. The CF-1 mouse could perhaps serve as a model for these groups.

Although doramectin and moxidectin were not tested in CF-1 mice, these compounds have been tested in groups of animals that are thought to be
similarly sensitive. It is known that, in neonatal rodents, the blood-brain barrier is not completely developed. The results of multigeneration reproductive toxicity studies might provide some information on the neurotoxic effects of avermectins in animals that allow this class of compounds to pass relatively unhindered across the blood-brain barrier. The view is supported by the findings of reproductive toxicity studies with abamectin and ivermectin. No-observed-effect levels (NOELs) for neurotoxicity are of a similar order in CF-1 mice dosed with abamectin and in neonatal rats exposed to abamectin or ivermectin via the maternal milk (4; Annex 1, reference 92). However, because the test systems used to assess the neurotoxicity of this group of compounds are of uncertain sensitivity and because doramectin and moxidectin were not tested in CF-1 mice, the Committee concluded that the safety factor normally applied in studies of this kind should be doubled in calculating the ADI so as to allow for the possibility that some sensitive human populations may exist (see pages 13 and 18).

2.3 **Microbiological assessment of residues in food**

At its present meeting, the Committee considered a working paper that had been prepared by Dr C.E. Cerniglia on the microbiological assessment of veterinary drug residues in food (8) in which a number of issues were addressed relating to methods of testing and assessing microbiological risk. Since the Committee recognized that these issues would benefit from widespread discussion, it recommended that the paper should be distributed to interested organizations and governments for comment. Any comments received would be taken into account when this subject was considered again at the next meeting on residues of veterinary drugs in food.

2.4 **Consideration of the total intake of substances used as veterinary drugs**

The Committee recognized that the possible impact of veterinary medicines and animal production aids on the environment is giving rise to increasing concern. Indeed, regulatory authorities already require information on this matter in submissions. The terms of reference of the Joint FAO/WHO Expert Committee on Food Additives are limited to the assessment of the effects of the consumption of residual amounts of veterinary drugs present in human food of animal origin. For this reason, the entire ADI for a compound is frequently allocated to such residues.

It is, however, possible to recognize circumstances in which the consumer may be exposed simultaneously to residues of a substance used both as a crop protection agent and as a veterinary drug. In this case the ADI should apply to both uses.

Multiple exposure might also result from the use of a drug in intensive farming, when some of the ingested dose is excreted chemically intact
or as active metabolites and is then spread on land or recycled as food for other species. A production aid could conceivably be used simultaneously in a large proportion of the intensive-farming industry. If it is excreted largely unchanged, is chemically stable in the environment, and enters the food chain or groundwater, it will be consumed by the population and in this way increase the total intake of the substance.

The importance of adequately assessing dietary intakes of food additives, residues of veterinary drugs and contaminants was recognized by the FAO/WHO Expert Consultation held in Geneva in March 1995 (1). In addition, the indirect exposure of humans to xenobiotics through the environment is already a major aspect of WHO’s environmental health activities.

The Committee wished to draw the attention of FAO and WHO to the fact that such aspects of consumer safety were not currently considered in its reports. While recognizing that the evaluation of this additional potential source of contamination of the diet had implications in terms both of the resources required and of the MRLs which it sets, the Committee requested FAO and WHO to consider how this matter could be investigated.

2.5 Chemical analysis of residues – effect of analytical methodology on assignment and monitoring of MRLs

Loss or apparent gain of target drug may occur during the processes of extraction from the analytical test material, purification of the extract, and determination. The extent of such analytical variation depends on the concentration of the analyte and/or the analytical procedure used, as well as on the recovery of the analyte, which can range from 30% to an apparent value in excess of 100%. Where an analytical procedure incorporates an internal standard, the outcome of the analysis is the total amount of the target drug because the fractions of the analyte and the internal standard lost during the analytical procedure are the same. Thus, different fully validated analytical procedures can deliver different analytical results on the same test material.

The consequence of variations in the recovery of an analyte is that the concentration of drug in the matrix is under- or overestimated; this can have a significant effect on the allocation of MRLs based on an assigned ADI value, and on the results of subsequent monitoring.

This factor has not been specifically addressed in the allocation of MRLs at previous meetings of the Committee. Nevertheless, it should be recognized that the assignment of an MRL to an analyte in a particular tissue sample is based on the assumption that there is complete (i.e. 100%) recovery of the analyte. Where complete recovery is not achieved by a particular method, the analytical results should be corrected to 100% to determine whether the residue is within, or exceeds, the MRL.

The Committee requested that sponsors provide both uncorrected and corrected analytical data for evaluation.
2.6 Residues at the injection site

At its thirty-eighth meeting (Annex 1, reference 97), the Committee expressed its concern about the relatively high levels of residues that may occur at injection sites, which may be located in non-discarded edible tissues. The Committee noted that, for the safety assessment of residues of drugs administered intramuscularly or subcutaneously (e.g. as implants), information on drug dose, formulation and time elapsed since injection was necessary. At its forty-second meeting (Annex 1, reference 110), the Committee reiterated the need for pharmacokinetic data on persistent residues at the injection site resulting from the use of long-acting formulations. At its present meeting, the Committee noted that several of the drugs being evaluated had residue levels at the injection site that are close to, and may even exceed, the MRL at practical withdrawal periods. Information has been provided on the persistence of these residues at the injection site; however, the Committee considered it desirable to standardize sampling procedures at the injection site in order to minimize the variability of the information obtained for review at future meetings.

The Committee recommended the adoption of the sampling procedure currently used by both the Committee for Veterinary Medicinal Products (CVMP) Working Party on the Safety of Residues (9) in the Commission of the European Communities and the Food and Drug Administration in the United States (10). The injection site is permanently marked, and the drug is then administered at its centre. At sampling, approximately 500 g of tissue, including the injection site, is removed for homogenization. The sample should have the general form of a cylinder of the following general dimensions:

- For intramuscular injections: 10 cm diameter and 6 cm depth.
- For subcutaneous injections: 15 cm diameter and 2.5 cm depth.

When tissue is taken from an intramuscular injection site for use in residue studies, care must be taken to ensure that, where possible, the needle track, the area of drug release and any area of tissue reaction are included in the sample. This is particularly important when long needles have been used. The experimental procedures should simulate those used in practice, and skin should be included when it is normally consumed.

3. Comments on residues of specific veterinary drugs

The Committee considered for the first time three anthelmintic agents, one antimicrobial agent and one antiprotozoal agent. It reconsidered three anthelmintic agents and three antimicrobial agents. The recommendations made with regard to these compounds are summarized
in Annex 2, while details of further toxicological studies and other information requested are given in Annex 3.

Toxicological monographs or monograph addenda were prepared on all of the compounds considered in this section except abamectin and oxytetracycline. Residues monographs were prepared on all the substances.

3.1 Anthelmintic agents

The Committee considered abamectin and doramectin, which belong to the avermectin class of compounds, and moxidectin, which belongs to the milbemycin class of compounds. Doramectin and moxidectin share a number of structural similarities with each other and with abamectin and ivermectin, which was previously evaluated at the thirty-sixth and fortieth meetings of the Committee (Annex 1, references 91 and 104). The structures of these compounds are shown in Fig. 1.

Figure 1
Chemical structures of doramectin, ivermectin, abamectin and moxidectin

[Chemical structures of doramectin, ivermectin, abamectin, and moxidectin are depicted here.]

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The Committee also considered the benzimidazoles fipronil, fenbendazole and oxfendazole, which were previously evaluated at the thirty-eighth meeting (Annex 1, reference 97).

3.1.1 Abamectin

Abamectin had not previously been evaluated by the Committee.

Abamectin is a fermentation product of *Streptomyces avermitilis* that is used both as a pesticide and as an anthelminthic drug in animals. Its structure is shown in Fig. 1.

The Committee considered data on the metabolism of abamectin and the depletion of residues of this drug from the edible tissues of cattle.

**Toxicological data**

Abamectin has been evaluated toxicologically by two Joint FAO/WHO Meetings on Pesticide Residues, held in 1992 and 1994 (11, 12). Toxicological monographs were published after both meetings (4, 13). An ADI of 0–0.2 μg per kg of body weight was established in 1994 based on a no-observed-adverse-effect level of 0.12 mg per kg of body weight per day for pup toxicity in a reproductive toxicity study in rats. A safety factor of 500 was applied because of concern about the teratogenicity of the Δ-8,9 isomer, a photolysis product that has been detected as a residue in plants.

**Metabolism data**

The metabolism of radiolabelled avermectin B₁₉, the major component of abamectin, was studied in rats and cattle. The metabolic profiles were similar in these two animal species. Avermectin B₁₉ was the major component of the total residues found in the tissues of rats and cattle. In cattle injected subcutaneously with abamectin at 0.3 mg per kg of body weight, this metabolite accounted for at least 40% of the total residues in liver at 14 and 21 days after dosing and for 52%, 40% and 25% of those in fat at 7, 14 and 21 days respectively. The other residues detected in liver at 14 days after dosing were avermectin B₁₉ (1-5%) and polar metabolites, mainly 24-hydroxymethyl B₁₉ (22%). In fat, non-polar metabolites accounted for at least half of the total residues at 21 days after dosing. Of the total radioactivity in the liver, 86% was extractable at 14 days. All the radioactivity in fat was extractable at 21 days.

**Pharmacokinetic data**

Radiometric studies showed that abamectin is well absorbed after subcutaneous administration. In cattle, the plasma concentration of radioactivity peaked at 1–2 days after dosing. In rats and cattle, the radioactivity was mainly eliminated in faeces; at 7 days after dosing, the percentage of the dose recovered in faeces was 70% in rats and 50% in cattle.
Table 1
Total residues expressed as abamectin equivalents (μg/kg) in cattle given a single subcutaneous injection of 0.3 mg of radiolabelled abamectin per kg of body weight

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Residues</th>
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<tr>
<td></td>
<td>Muscle</td>
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<td>Kidney</td>
<td>Fat</td>
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<td>619</td>
<td>143</td>
<td>444</td>
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<tr>
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<td>&lt;4⁵</td>
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</tbody>
</table>

* Injection site.

Residue data
The Committee considered a residue-depletion study in cattle given a single subcutaneous dose of 0.3 mg of radiolabelled abamectin per kg of body weight. The study indicated that the depletion rates of residues in kidney, liver and muscle between 7 and 35 days after dosing were similar. Apart from the injection site, residues were highest in liver at 7 and 14 days after dosing (Table 1).

Two residue studies using unlabelled abamectin were carried out in cattle. Animals were injected subcutaneously with abamectin at 0.2 or 0.3 mg per kg of body weight. The residues of avermectin B₁₃a were measured by a high-performance liquid chromatography (HPLC) method with fluorescence detection, which had a quantification limit of 5 μg/kg and a detection limit of 1 μg/kg.

In both studies, residue levels were highest in liver and fat at all sampling times. The depletion rates of residues in these two tissues were similar. In cattle given abamectin at a dose of 0.2 mg per kg of body weight, the average residue levels of avermectin B₁₃a in liver, kidney and fat were 53, 13 and 78 μg/kg, respectively, at 21 days after dosing, <10 μg/kg at 35 days after dosing, and 2 μg/kg at 42 days after dosing. In cattle administered a dose of 0.3 mg per kg of body weight, the average residue levels of avermectin B₁₃a were 30 μg/kg for liver and fat and <10 μg/kg for muscle and kidney at 20 days after dosing, and <10 μg/kg for liver and fat at 25 days after dosing.

In both studies, the mean residue level of avermectin B₁₃a at the injection site at 35 days after dosing was about 550 μg/kg.

Analytical method
Abamectin residues can be measured by the HPLC method with fluorescence detection. The method measures avermectin B₁₃a, which is
separated by solvent extraction and converted into a fluorescent derivative. The method is applicable to muscle, liver, kidney and fat, and has a quantification limit of 5 μg/kg and a detection limit of 1 μg/kg. It satisfies the requirements for accuracy and precision for each of these tissues. The average recoveries for the different tissues were in the range of 70-90% and the coefficients of variation were below 15%. This method is specific to avermectin B₁₂.

Maximum Residue Limits
In considering its decision on the MRLs for abamectin, the Committee took the following factors into account:

• The ADI of 0-0.2 μg per kg of body weight, which was established by a Joint FAO/WHO Meeting on Pesticide Residues (12). This would result in a maximum daily intake of residues of 12 μg for a 60-kg person.
• Liver and fat are considered to be the appropriate target tissues.
• Abamectin used as a veterinary drug is intended solely for use in beef cattle.
• No bound residues of abamectin are formed in fat tissue and such residues account for less than 15% of total residues in liver.
• Avermectin B₁₂ is considered to be the appropriate marker residue.
• Avermectin B₁₂ accounts for 42% of the total residues in liver and 25% of those in fat at 21 days after dosing.
• The quantification limit of the analytical method is 5 μg/kg.

The Committee noted that a Joint FAO/WHO Meeting on Pesticide Residues had evaluated abamectin residues resulting from pesticide use in 1992 (11). The ADI established at the Joint FAO/WHO Meeting on Pesticide Residues in 1994 was 0-0.2 μg per kg of body weight; a safety factor of 500 was used because of the teratogenicity of the Δ-8,9 isomer, which is not produced when abamectin is used as a veterinary drug (12).

MRLs have been recommended by the Joint FAO/WHO Meeting on Pesticide Residues for abamectin in some vegetables and fruits (14). Some MRLs have also been recommended for cattle and goat meat and milk to take into account possible contamination of these foods resulting from the ingestion of abamectin-treated vegetables and fruits by these animals.

The MRLs recommended by the Joint FAO/WHO Meeting on Pesticide Residues for abamectin in cattle and goats were 0.01 mg/kg for muscle, 0.005 mg/kg for milk (both species) and 0.05 and 0.1 mg/kg respectively for offal (14). From these values, the theoretical maximum daily intake of abamectin residues from these two species, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, and 1.5 l of milk (Annex 1, reference 85), is significantly greater than the maximum ADI of 12 μg of abamectin for a 60-kg person.
In order to comply with the ADI, the Committee would have to recommend MRLs for cattle muscle, liver, kidney and fat different from those recommended by the Joint FAO/WHO Meeting on Pesticide Residues. For this reason, the Committee did not recommend MRLs for abamectin used as a veterinary drug and recommended that consultations should be held between representatives of the Committee and the Joint FAO/WHO Meeting on Pesticide Residues to resolve these differences.

3.1.2 Doramectin

Doramectin had not been previously reviewed by the Committee.

Doramectin is the fermentation product of a specific strain of Streptomyces avermitilis that is used as an endoparasitic agent in non-lactating cattle. Its structure is shown in Fig. 1.

Toxicological data

The Committee considered data from a range of studies on doramectin, including the results of studies on its metabolism, acute and short-term toxicity, reproductive and developmental toxicity, and genotoxicity.

The oral bioavailability of doramectin in rats, as measured by the plasma concentration of the drug and the area under the plasma concentration-time curve, was approximately six times greater when doramectin was administered in sesame oil than when the drug was administered in an aqueous vehicle or in the diet. Metabolism studies in rats, dogs and cattle revealed a similar spectrum of metabolites in the liver and faeces of each species, suggesting that laboratory animals are suitable models for testing the toxicity of doramectin.

Acute toxicity studies were carried out in rodents. When doramectin was administered orally in an aqueous vehicle, the LD₉₀ values were in the range 500–2000 mg per kg of body weight in rats and greater than 2000 mg per kg of body weight in mice. When the drug was given in a sesame oil vehicle, the oral LD₉₀ values were 50–200 mg per kg of body weight in rats and 75–500 mg per kg of body weight in mice. These marked differences in acute toxicity reflect the enhanced absorption of doramectin when administered as an oily preparation. A further investigation in mice showed that the acute toxicities of orally administered doramectin and ivermectin were similar and that both compounds were less acutely toxic than abamectin or moxidectin. Toxic signs were indicative of effects on the central nervous system, since doramectin, like abamectin and other drugs in this class, affects γ-aminobutyric acid (GABA)-sensitive neurons, which can lead to neurotoxicity, as shown by tremors, ataxia and gait abnormalities.

Short-term administration of doramectin in the diet to mice was associated with clinical signs of neurotoxicity, and there was evidence of minor toxic effects on the liver and kidney at and above doses of 100 mg per kg of body weight per day. Plasma drug levels were dose-related,
although the relationship was non-linear up to 100 mg per kg of body weight per day and reached a plateau at doses greater than 100 mg per kg of body weight per day, which suggests that a threshold exists for the absorption of doramectin from the feed. The highest plasma concentration was 3.9 μg/ml.

A number of short-term toxicity studies were carried out in rats for periods of up to 3 months. Administration of doramectin in the diet at doses of 30 mg per kg of body weight per day and above resulted in markedly reduced food intake and body-weight gain, as well as severe neurotoxicity, which necessitated the early killing of some animals. Other findings at the same doses were atrophic changes in hepatocytes and lymphoid organs and nephrosis. Minor increases in liver weight were seen at 20 mg per kg of body weight per day in the diet, but hepatic morphology was unaffected at this dose. The maximum plasma drug level attained in these studies was 5.8 μg/ml. In a 1-month study in which doramectin was given in the diet, the NOEL in rats was 10 mg per kg of body weight per day.

In two studies, rats were given doses of doramectin by gavage for up to 3 months. Signs of central nervous system toxicity were observed at the highest dose of 10 mg per kg of body weight per day. At doses of 5 mg per kg of body weight per day and above, there were increases in liver weight, while kidney weight was increased at 8 mg per kg of body weight per day. However, there were no concomitant pathological changes and all other parameters were unaffected. Plasma drug levels reached a peak of 3.2 μg/ml. The NOEL in gavage studies in rats was 2 mg per kg of body weight per day.

When dogs were fed doramectin in the diet for periods of up to 36 days, the only treatment-related findings were mydriasis and reduced body weight at a dose of 4 mg per kg of body weight per day. The highest plasma drug level was 0.88 μg/ml. The NOEL in this study was 2 mg per kg of body weight per day.

In three studies in dogs, which received doramectin by gavage in a sesame oil vehicle for up to 92 days, body weights were depressed at 4 mg per kg of body weight per day and clinical signs of central nervous system toxicity were noted at 2 mg per kg of body weight per day and above. The most sensitive indication of a drug-related effect was mydriasis, which was seen at doses as low as 0.3 mg per kg of body weight per day. No other toxic effects were observed. The maximum plasma drug level in these studies was 3 μg/ml. The NOEL was 0.1 mg per kg of body weight per day.

Reproductive toxicity studies in rats revealed treatment-related deaths among pups during the early postnatal period and a reduction in body-weight gain of pups throughout the lactation period. These effects were observed at doses as low as 3 and 1 mg per kg of body weight per day, respectively. Doramectin was shown to be readily excreted in the milk of
lactating rats following administration by gavage and, as compared with adult animals, higher drug levels were attained in the brain of neonates, suggesting greater penetration through the incompletely formed blood-brain barrier in newborn rats. The NOEL was 0.3 mg per kg of body weight per day, based on toxicity in neonatal animals.

Administration of doramectin by gavage to pregnant mice and rats did not result in fetal abnormalities. The only drug-related finding was a slight increase in embryo mortality in mice given 6 mg per kg of body weight per day. The NOEL in mice was 3 mg per kg of body weight per day, while in rats there were no adverse effects at the highest dose of 6 mg per kg of body weight per day. In rabbits given 3 mg per kg of body weight per day during pregnancy, there was significant maternal toxicity, and cleft palate was observed in three fetuses from one of 20 litters; one fetus exhibited phocomelia, syndactyly and coelosomia. Ossification of pubic bones was delayed at doses of 1.5 and 3 mg per kg of body weight per day. In a dose-ranging study, a dose of 6 mg per kg of body weight per day caused severe maternal toxicity and embryotoxicity. The NOEL for maternal toxicity in rabbits was 0.75 mg per kg of body weight per day.

In view of the negative results in a range of in vitro genotoxicity assays and a micronucleus test in mouse bone marrow, the Committee concluded that doramectin was not genotoxic.

The Committee noted the close structural similarities between doramectin and abamectin, the only difference being the presence of a cyclohexyl group at the C-25 position in the doramectin molecule rather than an isopropyl or isobutyl group in the case of abamectin B₉₅ and abamectin B₉₆, respectively. The available metabolic data suggest that the biotransformation of doramectin and abamectin follows a similar pathway. Extensive toxicological tests have been conducted on both compounds and the Committee reviewed several aspects of their comparative toxicology. Both compounds exert a pharmacological effect on the central nervous system through effects on GABA-sensitive neurons, which results in a range of neurotoxic signs such as mydriasis, tremors, ataxia and gait abnormalities. In studies in which repeated doses are given, the compounds have been associated with few adverse toxicological effects, reduced body-weight gain and minor toxic effects on the liver being the most common. Toxicity in neonates is the most sensitive indicator in reproductive toxicity studies with both compounds. Neither compound is considered to have any genotoxic activity. Carcinogenicity studies with abamectin were negative at maximum tolerated doses in mice and rats. In view of the chemical, biochemical and toxicological similarities, the Committee concluded that it was unnecessary to request data from long-term toxicity and carcinogenicity studies on doramectin.

The Committee considered that the most relevant effect for the safety evaluation of residues of doramectin was the effect on the mammalian
nervous system. An ADI of 0-0.5 µg per kg of body weight was established, based on a NOEL of 0.1 mg per kg of body weight per day for mydriasis in a 3-month gavage study in dogs. A safety factor of 200 was applied because the test systems used to assess the neurotoxicity of doramectin were of uncertain sensitivity (see section 2.2). The ADI provides an adequate margin of safety for neonatal toxicity in rats and developmental toxicity in rabbits.

Pharmacokinetic data
Doramectin was equally well absorbed from intramuscular and subcutaneous injection sites. Most of the dose (>86%) was eliminated by day 14 in the faeces, <1% being eliminated in the urine.

Metabolism data
The metabolism of doramectin was studied in cattle, rats and dogs. Biotransformation was similar in all three species. Unchanged drug was the major component of residues in all tissues; its concentration was linearly related to the concentration of total residues in liver and fat. Three minor metabolites were identified in liver, fat and faeces. These metabolites were more polar than doramectin and were the result of O-demethylation in the distal saccharide ring, of hydroxylation of the 24-methyl group, and of a combination of these biotransformations. More than 90% of the residues were readily extracted from the tissues.

Residue data
Two residue-depletion studies were carried out in cattle using 3H-labelled doramectin at the recommended parenteral dose of 0.2 mg per kg of body weight. The concentrations of total residues and unchanged drug were measured in both edible tissues and the injection site. In the first study, the dose was administered as a subcutaneous injection, and samples were taken between days 21 and 35. In the second study, the dose was given by intramuscular injection and samples were taken between days 7 and 42. The highest levels of residues in edible tissues were found in fat (23-551 µg/kg) and liver (24-470 µg/kg). The depletion of total residues and unchanged doramectin was nearly linear (semi-logarithmic plot) in all tissues over the sampling periods, with the exception of liver in the second study, which was linear only from days 14 to 42. Total residues at the injection site were approximately 2.5 mg/kg at day 7 and fell to <1 mg/kg by day 35. The rate of depletion was essentially the same for total residues and unchanged drug in liver and fat, elimination half-lives varying between 5.6 and 8.1 days. When the results of both studies were combined, the mean percentages of unchanged drug in the total residues were 55% in liver and 80% in fat.

Three studies were available in which unlabelled doramectin was given to cattle at the recommended dose of 0.2 mg per kg of body weight by
either subcutaneous or intramuscular injection. Residue concentrations were slightly higher in the tissues of the cattle given the subcutaneous dose than in those injected intramuscularly. Levels of residues of parent drug were highest at the injection site and in fat and liver, and lowest in muscle and kidney. At 35 days after dosing by the subcutaneous and intramuscular routes, the mean concentrations were, respectively, 930 and 177 µg/kg at the injection site, 57 and 37 µg/kg in fat, 29 and 27 µg/kg in liver, <3 and <2 µg/kg in muscle, and <5 and 3 µg/kg in kidney. When all the values for the three studies were plotted for liver and fat, the values for the regression line reached 100 µg/kg and 10 µg/kg at 19 days and 40 days for liver, and 23 days and 43 days for fat, respectively. The concentration of total residues at the injection site, as measured on day 35, was much lower in animals given radiolabelled drug by the subcutaneous or intramuscular route (7 and <32 µg/kg respectively) than the concentration of residues of parent drug in animals treated with unlabelled drug (930 and 177 µg/kg respectively). There were wide variations in the concentrations of residues at the injection site in all studies.

Analytical methods
An analytical method using HPLC was suitable for monitoring residues of parent drug both in edible tissues and at the injection site. The method was further modified to include mass spectrometry for confirmatory purposes. The analytical method used by the sponsor was based on the extraction of doramectin from tissue homogenate or fat and its subsequent conversion into a chemically stable fluorescent aromatic derivative. The method was specific for doramectin and gave good chromatographic separation from other avermectins and milbemycins, including ivermectin, abamectin and moxidectin. Sensitivity was also good, the quantification limit being 2.5 µg/kg for liver, kidney, muscle and injection site tissue and 5 µg/kg for fat. The assay was linear up to 400 µg/kg. The percentage recovery of doramectin from liver and fat was high (>80%) and the accuracy (>90%) and precision (10% or lower, expressed as the coefficient of variation) of the method were also satisfactory.

Doramectin residues were confirmed by an analytical method based on HPLC and mass spectrometry. The analysis of tissues fortified with 25–250 µg/kg doramectin was successfully carried out, as was that of tissues of animals treated with the drug, where residue concentrations were predetermined to be in the range 50–85 µg/kg. Ivermectin did not cause any interference with the structural confirmation of doramectin by HPLC and mass spectrometry.

Maximum Residue Limits
In reaching its decision on MRLs for doramectin, the Committee took into account the following factors:
An ADI of 0.0–0.5 µg per kg of body weight was established. This would result in a maximum ADI of 30 µg for a 60-kg person.

The drug is intended only for use in non-lactating cattle.

The target tissues are fat and liver.

The parent drug is the marker residue and accounts for 55% of the total residues in liver, 80% in fat, 70% in muscle and 75% in kidney.

Less than 10% of the residues are bound.

The drug is administered as a single dose.

The quantification limits of the analytical method are 2.5 µg/kg for muscle, liver and kidney, and 5 µg/kg for fat.

The Committee recommended the following MRLs for cattle: 10 µg/kg for muscle, 100 µg/kg for liver, 30 µg/kg for kidney and 150 µg/kg for fat, expressed as parent drug. Based on these values for the MRLs, the maximum theoretical intake would be 33 µg per day (Table 2). This would be compatible with a maximum ADI of 30 µg for a 60-kg person.

Data for doses above 0.2 mg per kg of body weight, whether administered as single or multiple doses, were not available. The Committee noted the high concentration of residues at the injection site in cattle over a 35-day period after subcutaneous or intramuscular administration of the unlabelled drug at the recommended dose.

### 3.1.3 Moxidectin

Moxidectin had not been previously evaluated by the Committee.

Moxidectin is an antiparasitic drug that is used to control a range of internal and external parasites in sheep, cattle and deer. Its structure is shown in Fig. 1.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intake (µg/kg) doramectin equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>10</td>
<td>14(^{c})</td>
<td>4</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>182(^{c})</td>
<td>18</td>
</tr>
<tr>
<td>Kidney</td>
<td>30</td>
<td>40(^{a})</td>
<td>2</td>
</tr>
<tr>
<td>Fat</td>
<td>150</td>
<td>188(^{f})</td>
<td>9</td>
</tr>
</tbody>
</table>

| Total       | 33                      |

\(^{a}\) Expressed as parent drug.

\(^{b}\) Based on a daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat.

\(^{c}\) The marker residue accounted for 70% of the total residues in muscle.

\(^{d}\) The marker residue accounted for 55% of the total residues in liver.

\(^{e}\) The marker residue accounted for 75% of the total residues in kidney.

\(^{f}\) The marker residue accounted for 80% of the total residues in fat.
Toxicological data

The Committee considered data from pharmacodynamic, pharmacokinetic, metabolism, acute and short-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity and teratogenicity studies.

The pharmacokinetics and metabolism of moxidectin were studied in rats, sheep and cattle. After oral administration to sheep, about 20% of the dose was absorbed. The drug, which is very lipophilic, was found at high levels in fat, but at much lower levels in other tissues. It was excreted in the milk. After oral administration to rats, the major compound recovered in faeces was the parent drug, while small amounts of hydroxylated metabolites were found in the liver and faeces. Hydroxylated metabolites were also found in the liver and faeces of sheep and cattle given oral doses of the drug.

Orally administered moxidectin was found to be moderately toxic in acute toxicity studies in mice and rats, with LD₅₀ values of the order of 50-100 mg per kg of body weight.

In a 28-day toxicity study in mice in which moxidectin was administered in the feed, signs of toxicity included tremors and hypersensitivity to touch. All mice given the highest dose of 32 mg per kg of body weight per day died. The NOEL in this study was 6.9 mg per kg of body weight per day.

Rats given up to 31 mg of moxidectin per kg of body weight per day in a 28-day feeding study developed ataxia, tremors, salivation, piloeruction and diuresis. At 23 mg per kg of body weight per day and above, dose-related diffuse atrophy of the liver, kidneys, heart, spleen, adrenals, testes, epididymides and ovaries was seen. A NOEL was not identified in this study because hypersensitivity to touch was noted in the group receiving the lowest dose (12 mg per kg of body weight per day) at days 2 and 3. Similar findings were noted in a 13-week feeding study in rats, in which the NOEL was 3.9 mg per kg of body weight per day.

In a 28-day oral toxicity study in dogs given up to 4 mg of moxidectin per kg of body weight per day, animals given the highest dose developed anorexia, ataxia, prostration and diarrhoea until the dose was reduced to 1.25 mg per kg of body weight per day. The NOEL was 0.5 mg per kg of body weight per day. In a 90-day feeding study in dogs, lacrimation, tremors, salivation and slight ataxia were noted in animals given the highest dose of 1.6 mg per kg of body weight per day. No major histopathological changes were observed in this study. The NOEL was 0.3 mg per kg of body weight per day. No signs of toxicity were noted in a 52-week feeding study in dogs. The NOEL was 1.15 mg per kg of body weight per day, the highest dose used.

A range-finding single-generation reproductive toxicity study was performed in rats, in which moxidectin was administered at doses of up to approximately 10 mg per kg of body weight per day for a 9-week period before mating and during gestation and lactation. None of the
offspring of animals in the highest-dose group survived. Survival rates were decreased in the progeny of animals given lower doses, but there were no findings that could be attributed to effects on fertility. The NOEL in this study was 0.4 mg per kg of body weight per day. In a three-generation reproductive toxicity study in rats given doses of up to 0.83 mg per kg of body weight per day for a 70-day period before mating, there were no effects on mortality or on fertility except at the highest dose, where slight reductions in male body weights and significant reductions in pup survival were seen. The NOEL was 0.4 mg per kg of body weight per day.

In a teratogenicity study in rats dosed at 10 or 12 mg moxidectin per kg of body weight per day, there was evidence of both maternal toxicity and fetotoxicity, as shown by increased incidences of cleft palate and wavy or incompletely ossified ribs. There was no evidence of teratogenic effects, and the NOEL in this study was 5 mg per kg of body weight per day. In a teratogenicity study in rabbits, there was evidence of maternal toxicity at 5 and 10 mg per kg of body weight per day, but no evidence of fetotoxicity or teratogenicity. The NOEL in this study, based on maternal effects, was 1 mg per kg of body weight per day.

In a long-term toxicity/carcinogenicity study in CD-1 mice, moxidectin was administered in the diet at concentrations equal to 2.5, 5.1 or 12 mg per kg of body weight per day for 2 years. After 9 weeks, the highest dose was reduced to 7.9 mg per kg of body weight per day because of toxic effects that included deaths, hunched posture, decreased activity, tremors and laboured breathing. There were no effects on haematological parameters and no increases in the incidence of any types of tumour were observed. The NOEL was 5.1 mg per kg of body weight per day.

A 2-year toxicity/carcinogenicity study was conducted in Sprague-Dawley rats, which were given moxidectin in the diet at concentrations equal to 0.8, 3.2 or 9.8 mg per kg of body weight per day. After 8 weeks, the highest dose was reduced to 5.1 mg per kg of body weight per day because of signs of toxicity that included hunched posture, tremors, hyperactivity, urine-stained fur and hypersensitivity to external stimuli. At the end of the 2-year period there was no evidence of toxicity and no increased incidence of any type of tumour. The NOEL was 5.1 mg per kg of body weight per day.

The Committee concluded that moxidectin has no carcinogenic potential. Moxidectin has been tested in bacterial mutation assays, in a forward mutation assay in Chinese hamster ovary cells, in a test for unscheduled DNA synthesis in primary rat hepatocytes, and in an in vivo cytogenetic assay in rat bone marrow. All gave negative results, and the Committee concluded that moxidectin had no genotoxic potential.

The Committee concluded that the most relevant effects for the toxicological evaluation of moxidectin were those observed in the 90-day study in dogs, where the NOEL was 0.3 mg per kg of body weight per
day. Based on this NOEL and using a safety factor of 200 to account for
the uncertain sensitivity of the test systems used to assess the
neurotoxicity of moxidectin (see section 2.2), the Committee established
an ADI of 0-2 µg per kg of body weight for moxidectin.

The ADI was rounded to one significant figure, consistent with accepted
rounding procedures (Annex 1, reference 91, section 2.7). This ADI
provides an adequate margin of safety for the effects noted in the
reproductive toxicity studies in rats.

Pharmacokinetic data
[¹¹C]Moxidectin was completely absorbed in cattle following
subcutaneous administration, peak blood concentrations being reached
within 7-8 hours; it was slightly less well absorbed (76% of the dose) in
sheep. When administered orally, about 20% of the drug was absorbed in
sheep and rats, peak blood concentrations being reached within 10 hours.
The half-life of moxidectin is about 80 hours in cattle and sheep.

The excretion of moxidectin was measured in cattle and rats, using the
radiolabelled compound. The primary route of excretion was via the
faeces, with <3% and <1% of the radioactivity being excreted in the urine
in cattle and rats, respectively.

Metabolism data
Moxidectin was metabolized in a similar manner in cattle and sheep. The
principal component of the residues was parent drug, which accounted
for 75-95% of total residues in fat and 40-90% of those in other edible
tissues at 7-28 days after dosing in cattle and at 7 days after dosing in
sheep. In rats, parent drug was also the major residue. Two minor
residues were found in cattle and sheep, and there were one similar and
two different minor metabolites in rats. In all studies, the majority
(86-95%) of the total radiolabelled residues were extractable, indicating
that only a small fraction was bound.

Residue data
Residue levels in the edible tissues of cattle and sheep were determined
following administration of [³H]moxidectin or [¹¹C]moxidectin at the
recommended dose or at twice the recommended dose. The drug was
administered subcutaneously to both species; in addition, cattle were
treated with a pour-on preparation, while sheep were given an oral drench
preparation. The study indicated that:

- Residue depletion was similar in cattle and sheep, and was
  independent of the route of administration and the formulation.
- The residues were lowest when the drug was administered as a pour-
  on preparation.
- The residues were highest in fat and lowest in muscle. For example, in
  steers given a subcutaneous dose of 0.2 mg per kg of body weight, the
levels in fat were 424 and 186 µg/kg at 14 and 28 days, respectively, as compared with 10 and 4 µg/kg in muscle. There was little difference between the residue concentrations in omental and back fat.

Fat and liver were recommended as the target tissues and unchanged drug as the marker compound for all tissues.

Ten residue-depletion studies were carried out using commercial preparations of moxidectin. Cattle and sheep were treated with a subcutaneous preparation, cattle and deer with a pour-on preparation, and sheep with an oral drench. The residues of unchanged moxidectin in the edible tissues and milk were measured by an HPLC method after periods ranging from 7 to 50 days. In all cases, the residues were low (usually <50 µg/kg) in muscle, liver, kidney and milk, but much higher in omental and back fat. Over a 14–28-day withdrawal period, the upper 99% confidence limits for the concentration of moxidectin in fat were 88–438 µg/kg, and declined slowly. No attempt was made to measure the residues in the tissue in the immediate vicinity of the pour-on application.

The residues at the site of subcutaneous injection were measured using [³H]moxidectin and unlabelled moxidectin. The injection-site samples were either 15 cm in diameter and 2.5 cm in depth (for the radiolabelled drug) or about 500 g in weight (for the unlabelled drug). There was wide variation in the results in different animals, and residue concentrations were lower (<10–79 µg/kg at 49 days) in animals treated with the radiolabelled drug than in those treated with the unlabelled drug (234–3190 µg/kg at 49 days), even though the dose of the unlabelled drug was half that of the radiolabelled drug.

Analytical methods

An HPLC method with fluorescence detection was developed for the determination of moxidectin in the edible tissues of sheep and cattle. Samples were extracted with organic solvents. The moxidectin residues were derivatized and, after further purification on small disposable chromatography columns, quantified on an HPLC column. The quantification limit was 10 µg/kg and the detection limit 1–4 µg/kg. Average recoveries were measured at three different laboratories and were 94–96%. The method, when applied to fat, was specific for moxidectin at the 250-µg/kg level in the presence of several of the most commonly used animal health products, namely ivermectin, monensin, levamisole, oxytetracycline, procaine benzylpenicillin, rofoxanide, sulfadimidine and trenbolone, at fortified levels of 50–200 µg/kg and fenbendazole at 20 mg/kg. A similar method was also used for deer tissues. Ivermectin was used as the internal standard, recoveries were in the range 70–90% and the quantification limit was 2 µg/kg. A liquid chromatography/mass spectrometry method was described for the confirmation of moxidectin at the 250-µg/kg level in fat.
Maximum Residue Limits
In reaching its decision on MRLs, the Committee took the following factors into account:

- The ADI of 0–2 μg/kg of body weight per day, which is equivalent to a maximum ADI of 120 μg for a 60-kg person.
- Fat and liver are the target tissues.
- The parent drug is the marker residue and accounts for 40% of the total residues in muscle, liver and kidney and 75% of the total residues in fat.
- Bound residues account for 5–15% of the total residues, and information was not available to enable them to be disregarded in the calculation of the MRLs.
- The quantification limit of the analytical method (10 μg/kg).
- The sponsors do not propose to make the drug available for use in lactating cows and cows in late pregnancy. Thus, residues in milk should not be taken into account.

The Committee recommended MRLs for moxidectin of 20 μg/kg for muscle, 100 μg/kg for liver, 50 μg/kg for kidney and 500 μg/kg for fat, expressed as parent drug, in cattle, sheep and deer. The recommended MRLs in deer are temporary, pending the receipt of further information on the marker residue in the edible tissues. This information is required for evaluation in 1998.

From these values for the MRLs, the maximum theoretical intake would be 79 μg per day (Table 3). This would be compatible with a maximum ADI of 120 μg for a 60-kg person.

The Committee noted the very high concentrations and great variation in the level of residues at the injection site in cattle over a 49-day period after dosing.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (μg/kg)(^a)</th>
<th>Estimate of total residues (μg/kg)</th>
<th>Theoretical maximum daily intake(^b) (μg/kg moxidectin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>20</td>
<td>50(^c)</td>
<td>15</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>250(^c)</td>
<td>25</td>
</tr>
<tr>
<td>Kidney</td>
<td>50</td>
<td>125(^c)</td>
<td>6</td>
</tr>
<tr>
<td>Fat</td>
<td>500</td>
<td>665(^c)</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>79</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as parent drug.
\(^b\) Based on a daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat.
\(^c\) The marker residue accounted for 40% of the total residues in muscle, liver and kidney.
\(^d\) The marker residue accounted for 75% of the total residues in fat.
3.1.4 *Febantel, fenbendazole and oxfendazole*

Febantel, fenbendazole and oxfendazole were previously evaluated at the thirty-eighth meeting of the Committee (Annex 1, reference 97).

A temporary ADI of 0–25 µg per kg of body weight was established for fenbendazole at that time, based on a NOEL of 5 mg per kg of body weight per day in a long-term toxicity/carcinogenicity study in rats and a safety factor of 200. Additional information on the mechanism of the observed increased incidence of liver tumours in female rats at high doses, including the results of an in vivo DNA binding study, was requested.

A temporary ADI of 0–10 µg per kg of body weight was established for febantel at the thirty-eighth meeting, based on a NOEL of 2 mg per kg of body weight per day in a two-generation reproductive toxicity study in rats and a safety factor of 200. Additional information on the genotoxic and carcinogenic potential of febantel, including the results of an in vivo DNA binding assay in rats, was requested.

A temporary ADI of 0–4 µg per kg of body weight was established for oxendazole at the thirty-eighth meeting, based on a NOEL of 0.7 mg per kg of body weight per day in a carcinogenicity study in rats and a safety factor of 200. Additional data from genotoxicity and teratogenicity studies were requested, including the results of an in vivo binding assay in rats.

**Toxicological data**

At its present meeting, the Committee considered the data available at the thirty-eighth meeting (Annex 1, reference 97), together with the results of an additional genotoxicity study on fenbendazole.

Febantel and fenbendazole have been tested in a range of genotoxicity assays, while oxfendazole has been tested only in an Ames test in which four strains of *Salmonella typhimurium* were used. The compounds consistently gave negative results in the Ames test, a test for DNA repair and in vivo cytogenetic assays. Fenbendazole, febantel and a metabolite (2-amino-5-phenylsulfanyl-2-benzimidazole) were weakly positive in the mouse lymphoma forward mutation assay in the presence of a metabolic activation system. Febantel, when tested at a sufficiently high dose to reduce fertility, was found to induce dominant lethal mutations in mice.

At its present meeting, the Committee noted that available data on the mode of action of benzimidazoles as inhibitors of the polymerization of tubulin to microtubulin, with consequent disruption of mitosis, together with the results of genotoxicity tests, do not provide any evidence of a direct interaction of these compounds with DNA. It therefore concluded that the positive results in some genotoxicity assays are likely to have been caused by an indirect mechanism, and that further studies on the binding of febantel, fenbendazole and oxfendazole to DNA are not required.
In addition, the Committee re-evaluated the long-term toxicity/carcinogenicity studies on all three compounds that were reviewed at the thirty-eighth meeting, and reached the conclusions given below.

**Febantel.** Combined long-term toxicity/carcinogenicity studies on febantel in rats and mice did not reveal increased incidences of any types of tumour. At its thirty-eighth meeting (Annex 1, reference 97), the Committee was of the opinion that higher doses could have been used in the carcinogenicity study in rats. However, in the group of rats given febantel at 500 mg/kg in the diet (equal to 40 mg per kg of body weight per day), decreases in body-weight gain, slight anaemia (in females only), increased liver weight, enlargement and vacuolization of liver cells, and significant increases in serum alkaline phosphatase activity were observed. These changes were not seen at the lower doses or in the control groups. At its present meeting, the Committee considered that these changes indicated that the dose levels used were sufficient for the carcinogenic potential of febantel to be assessed, and concluded that there was no evidence that it was carcinogenic in mice or rats.

**Fenbendazole.** In a lifetime study with an initial in utero phase, rats were dosed with fenbendazole at doses of 5, 15, 45 or 135 mg per kg of body weight per day. There were no increases in tumour incidence at 5, 15 or 45 mg per kg of body weight per day. At 135 mg per kg of body weight per day only a marginal increase in the incidence of liver tumours as compared with controls was observed. At the beginning of the lifetime study, the mean body weights of the highest-dose group (84 g for males and 79 g for females) were more than 40% lower than those of the controls (142 g for males and 123 g for females). Furthermore, the rats in the highest-dose group showed signs of toxicity at that time, including alopecia, icterus, diarrhoea and lethargy. The Committee considered that the results for this group could not be used for the assessment of the carcinogenicity of fenbendazole because the maximum tolerated in utero dose had been exceeded. However, proliferative liver lesions in the group receiving 45 mg per kg of body weight per day, described as hepatocellular hyperplasia and biliary cysts, which were not observed at lower doses, indicated that this dose level was high enough to permit an assessment of the carcinogenic potential of fenbendazole. On the basis of this study in rats and the negative results in the carcinogenicity study in mice reviewed at the thirty-eighth meeting (Annex 1, reference 97), the Committee concluded that there was no evidence that fenbendazole possesses carcinogenic potential.

**Oxfendazole.** The highest dose used in the rat carcinogenicity study was selected in the light of the results of a 3-month oral toxicity study in rats. In the latter study, increased mortality was observed at a dose level of 600 mg/kg in the diet (equal to 48 mg per kg of body weight per day for males and 50 mg per kg of body weight per day for females) and elevated alkaline phosphatase levels at a dose level of 200 mg/kg in the diet (equal to 17 mg per kg of body weight per day for males and 18 mg per kg of
body weight per day for females). A dose level of 100 mg/kg in the diet (equal to 7.4 mg per kg of body weight per day for males and 7.8 mg per kg of body weight per day for females) was selected as the highest dose in the carcinogenicity study. No carcinogenic effects were seen at any dose level in this study. At its present meeting, the Committee considered that histopathological findings of hepatocellular vacuolization in the liver of rats fed more than 2 mg per kg of body weight per day provided evidence of sufficient systemic exposure to oxendazole, and concluded that the study was adequate for assessing the carcinogenic potential of the compound. On the basis of this study and the negative results in the carcinogenicity study in mice reviewed at the thirty-eighth meeting (Annex 1, reference 97), the Committee concluded that there was no evidence that oxendazole possessed carcinogenic potential.

At its thirty-eighth meeting (Annex 1, reference 97), the Committee had requested an additional teratogenicity study in rabbits using oxendazole. This was not provided at the present meeting. Instead, the sponsor suggested that the available reproductive data in sheep were sufficient. The Committee concluded, however, that the study in sheep did not adequately explore the teratogenic potential of oxendazole, because it was designed to assess the safety of the drug in the target species.

Appraisal. The available data from reproductive and teratogenicity studies with each of the three drugs suggest that oxendazole is the most potent in producing embryotoxic effects. The data on fenbendazole suggest that the rabbit is the species most sensitive to the embryotoxic and teratogenic effects of these three compounds. The Committee therefore decided that an adequate teratogenicity study in rabbits with oxendazole was still required.

The Committee noted that, based on the NOELs identified at the thirty-eighth meeting (Annex 1, reference 97) and a safety factor of 100, ADIs of 0–20 and 0–50 μg per kg of body weight could have been established for febantel and fenbendazole, respectively. However, it also noted that febantel, fenbendazole and oxendazole have common metabolic routes, and that the residue data demonstrate that oxendazole is the major residue in food-producing animals following administration of these three compounds. Therefore, the Committee assigned a group temporary ADI of 0–4 μg per kg of body weight to febantel, fenbendazole and oxendazole based on the NOEL of 0.7 mg per kg of body weight per day for oxendazole identified at the thirty-eighth meeting and a safety factor of 200 (Annex 1, reference 97). The results of a teratogenicity study in rabbits, in which oxendazole is administered at sufficiently high doses for its teratogenic potential to be adequately explored, are required by the Committee for evaluation in 1998.

Residue data
At its thirty-eighth meeting, the Committee recommended temporary MRLs for febantel, fenbendazole and oxendazole of 100 μg/kg for
muscle, fat and kidney (cattle, sheep and pigs); 500 µg/kg for liver (cattle, sheep and pigs); and 100 µg/l for milk (cattle) (Annex 1, reference 97). The temporary MRLs were applicable to febantel, fenbendazole and oxfendazole individually or in combination. The MRL value was the sum of the concentrations of fenbendazole, oxfendazole and oxfendazole sulfone, calculated as oxfendazole sulfone equivalents.

At its present meeting, the Committee reviewed 17 residue-depletion studies in cattle, sheep and pigs in which various formulations of fenbendazole were used. The residues of fenbendazole, oxfendazole and oxfendazole sulfone were determined by a new analytical method. When fenbendazole is administered to an animal, it is reversibly oxidized to oxfendazole, and then either reduced to fenbendazole or irreversibly oxidized to oxfendazole sulfone. In this new method, an oxidative step using peracetic acid is included to convert any fenbendazole and oxfendazole into oxfendazole sulfone. The total residue of oxfendazole sulfone is then determined quantitatively, after separation by HPLC with fluorescence detection. The method has been validated by fortifying tissue samples (including milk) with a mixture of fenbendazole, oxfendazole and oxfendazole sulfone. The adequacy of the extraction procedure has been shown by tests in which fenbendazole, oxfendazole and oxfendazole sulfone were measured separately. The quantification limit of the HPLC method in all tissues, including milk, is 5 µg/kg; the method is linear in the range 5-1000 µg/kg in liver and milk and 5-200 µg/kg in kidney, fat and muscle.

Four residue-depletion studies were conducted in cattle, using different formulations of fenbendazole administered at a dose rate of 7.5 mg per kg of body weight. In the first study, groups of cattle (four per group) were slaughtered 5, 9, 14, 21 and 28 days following administration of a 10% suspension of the drug. Residues in liver were below the temporary MRL of 500 µg/kg at day 9, while those in other tissues were below the quantification limit of the assay. In the second study, groups of cattle were slaughtered 7, 11, 15, 18 and 21 days following administration of the 10% suspension. Residues in liver were well below the temporary MRL at day 7 and those in muscle, kidney and fat were either below the quantification limit or well below the temporary MRLs. In the third study, 22% granules were administered in the diet to cattle, which were slaughtered in groups (four per group) 5, 9, 14 and 21 days after dosing. Residues in all edible tissues were below the temporary MRLs at day 9. In the final study, 1.5% pellets were administered in the diet to cattle, which were slaughtered in groups (six per group) 7, 14 and 28 days after dosing. At day 14 after dosing, residue levels in liver were well below the temporary MRLs, and those in muscle, kidney and fat were below the quantification limit of the assay.

Two additional residue-depletion studies were conducted in cattle, using the 10% suspension and 1.5% pellet formulations. The dose rate in both studies was 7.5 mg per kg of body weight. The residues of fenbendazole
in milk were analysed both by an HPLC method in which fenbendazole, oxfendazole and oxfendazole sulfone were determined individually as well as by the new HPLC method with fluorescence detection. Total residues in milk were below the temporary MRLs of 100 μg/kg by day 5 and day 3 following administration of the 1.5% pellets and 10% suspension, respectively.

Four residue-depletion studies were conducted in sheep, two with a 2.5% suspension and two with a 250-mg bolus. Both formulations were administered at 5 and 10 mg per kg of body weight. The sheep were slaughtered in groups (four per group) 5, 9, 14 and 21 days after dosing; in two studies animals were also slaughtered at day 28. In all four studies the residues in liver at 14 days following drug administration were at or below the temporary MRLs, and those in other tissues were well below the temporary MRLs.

Two residue-depletion studies were reported in lactating sheep, one with a 2.5% suspension and one with a 250-mg bolus. Both formulations were administered at a dose rate of 5 mg per kg of body weight per day. The residues were analysed by the new HPLC method with fluorescence detection. Total residues in milk were below 100 μg/l at 3 days after dosing.

Five studies were conducted in pigs, one with the 1.5% pellets and the remaining four with a 4% powder. In the first and second studies, fenbendazole pellets and powder were administered once in the feed, at a dose rate of 5 mg per kg of body weight, respectively. In the remaining three studies, the powder formulation was given in the feed as follows: 3 mg per kg of body weight per day for 3 days; 1 mg per kg of body weight per day for 5 days; and 0.3 mg per kg of body weight per day for 15 days, respectively. Sampling times for the five studies were: 5, 10, 14 and 21 days; 5, 10, 14, 21 and 27 days; 5, 7, 10 and 14 days; 5, 7, 10 and 14 days; and 5, 7 and 10 days, respectively. All tissue residue levels were well below the temporary MRLs at all slaughter times.

**Maximum Residue Limits**

The Committee recommended that the MRLs for febantel, fenbendazole and oxfendazole should continue to be based on the temporary ADI of 0–4 μg per kg of body weight previously allocated to oxfendazole and that they should remain temporary. For a 60-kg person, this corresponds to a maximum daily intake of residues of febantel, fenbendazole and oxfendazole of 240 μg.

The recommended temporary MRLs, determined as the sum of oxfendazole, fenbendazole and oxfendazole sulfone and expressed as oxfendazole sulfone equivalents, for cattle, sheep and pigs are:

- Muscle, kidney and fat – 100 μg/kg
- Liver – 500 μg/kg
- Milk (cattle and sheep) – 100 μg/l.
These MRLs would result in a maximum daily intake of 240 μg of residues of febantel, fenbendazole and oxfendazole, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, and 1.5 l of milk (Annex 1, reference 85).

The Committee noted that, with the increasing production of goats in developing countries, residue data would be required for establishing MRLs in this species.

The results of ongoing residue-depletion studies on total residues of fenbendazole, oxfendazole and oxfendazole sulfone in cattle and sheep following the administration of febantel and oxfendazole are required for evaluation in 1998.

3.2 Antimicrobial agents

3.2.1 Ceftiofur

Ceftiofur had not been previously evaluated by the Committee.

Ceftiofur is a cefalosporin antibiotic that exhibits a broad spectrum of activity against both Gram-positive and Gram-negative bacteria, including β-lactamase-producing bacterial strains. It is bactericidal and inhibits bacterial cell wall synthesis in a similar fashion to other cefalosporins. Ceftiofur is used in the treatment of respiratory infections in cattle and pigs.

Toxicological data

A range of studies on ceftiofur and its primary metabolites were available for evaluation by the Committee, including data on pharmacokinetics and metabolism, acute and short-term toxicity, reproductive and developmental toxicity, genotoxicity, immunotoxicology and microbiology.

Ceftiofur is rapidly metabolized to desfuroylceftiofur. Following intramuscular administration in the rat, approximately 55% of the dose was excreted in the urine and about 30% in the faeces within the first 24 hours. Similar results were obtained in cattle. In a separate oral study in rats, approximately 55% of the dose was recovered in urine; the remainder was present in the faeces and the gastrointestinal tract.

Single oral doses of ceftiofur of up to 7800 mg per kg of body weight produced only minimal toxicity in the rat. Toxic signs associated with repeated oral doses in rats of up to 6000 mg per kg of body weight per day for 30 days were limited to haematological changes and diarrhoea. Oral doses of up to 300 mg per kg of body weight per day given to dogs for 91 days produced a reversible anaemia and thrombocytopenia. The NOEL for treatment-related haematopoietic effects in rats was 30 mg per kg of body weight per day.
In reproductive toxicity studies in rats, ceftiofur administered at dose levels of up to 1000 mg per kg of body weight per day had no adverse effects on fertility, reproductive performance or reproductive organs. Similarly, no treatment-related effects were observed in developmental toxicity studies in mice at doses of up to 4000 mg per kg of body weight per day or in rats at doses of up to 3200 mg per kg of body weight per day.

A variety of *in vitro* and *in vivo* genotoxicity assays covering a range of end-points were conducted with ceftiofur with and without metabolic activation with S-9 microsomal fraction and its metabolite furoic acid. All the assays were negative, with the exception of an *in vitro* chromosomal aberration assay in the absence of metabolic activation but only at concentrations at which cell division was inhibited. The Committee concluded that this finding, when taken in conjunction with the negative *in vivo* chromosomal aberration studies, was not of biological significance.

Carcinogenicity studies have not been performed on ceftiofur. However, the Committee noted that the drug showed no evidence of genotoxicity in a variety of assays and is not chemically related to known carcinogens. Furthermore, it is rapidly metabolized and its metabolites are not related to any known carcinogens. Neither neoplastic nor preneoplastic lesions were observed in 90-day feeding studies in rats, dogs and monkeys or in reproductive toxicity studies in rats involving exposure for periods of up to 160 days in which limited histopathological examinations were carried out. Recent reports indicate that non-genotoxic chemicals showing such a lack of toxicity are not associated with carcinogenicity in long-term (2-year) rodent studies. Under these circumstances, the Committee concluded that carcinogenicity studies were not necessary.

Long-term toxicity studies were not available. Even at doses exceeding several grams per kg of body weight per day for periods of up to 90 days, diarrhoea was the only major effect noted in rats. The Committee concluded that allowance could be made for the absence of long-term toxicity studies on ceftiofur by the application of an appropriate safety factor.

The potential immunotoxicity of ceftiofur has also been investigated. The Committee noted that penicillin antibodies do not recognize ceftiofur antigenic determinants and that exposure to metabolites of ceftiofur did not produce adverse reactions in guinea-pigs sensitized to penicillin. The Committee concluded that there is no risk of hypersensitivity reactions in humans to ceftiofur or its residues or metabolites at the anticipated level of exposure.

*Microbiological data*

The potential for adverse effects on the human gut flora was considered. *In vitro* minimum inhibitory concentration (MIC) data covering a wide range of animal and human bacterial species were submitted for
evaluation. A total of 58 strains commonly isolated from the human gastrointestinal tract were tested with ceftiofur and its metabolites. Ceftiofur was more active than its metabolites desfuroylceftiofur, 3,3’-desfuroylceftiofur disulfide and desfuroylceftiofur cysteine disulfide. The Committee recognized, however, that ceftiofur is not present as a residue because it is extensively and rapidly metabolized, with a plasma half-life of approximately 15 minutes in cattle and pigs. The lowest MIC50 value reported for desfuroylceftiofur cysteine disulfide was 2 μg/ml for Clostridium and Escherichia species.

In calculating an ADI based on antimicrobial activity, the Committee used the formula developed at its thirty-eighth meeting (Annex 1, reference 97):

\[
\text{Upper limit of temporary ADI} = \frac{\text{Concentration without effect on human gut flora (μg/ml)}}{\text{Fraction of oral dose bioavailable}} \times \frac{\text{Daily faecal bolus (g)}}{\text{Safety factor}} \times \frac{\text{Weight of human (60 kg)}}{150} \\
= \frac{2 \times 150}{0.1 \times 1 \times 60} = 50 \, \mu\text{g per kg of body weight}
\]

It took the following factors into account:

- Factors to account for the range of MICs needed to allow for sensitive bacteria, anaerobic environment, bacterial density and pH: the most relevant sensitive species were studied under conditions of high inoculum density. No adjustment was deemed necessary.
- Availability: the fraction of the dose available to the gut microflora was derived from studies of ceftiofur in humans which showed that the drug was rapidly metabolized.
- Variability among exposed individuals: the Committee noted that a substantial amount of data covering a variety of bacterial strains representative of the human gut microflora was available. In addition, it recognized that the other values selected for this calculation were already conservative and incorporated an adequate margin of safety. A safety factor of 1 was therefore adopted.

The Committee noted that the lowest NOEL based on toxicological studies was 30 mg per kg of body weight per day, which was observed in the 90-day study in dogs. It could establish an ADI of 0–60 μg per kg of body weight based on this NOEL and a safety factor of 500, which would include an additional safety factor of 5 to take account of the absence of long-term toxicity studies. However, the Committee noted that the microbiological end-point would give the lowest ADI and therefore established an ADI of 0–50 μg per kg of body weight based on this end-point.
Residue data

The Committee considered data on the metabolism of ceftiofur in rats, cattle and pigs, and on the depletion of ceftiofur residues from the edible tissues of cattle and pigs, as well as from cows' milk. Studies were conducted using both $[^{14}\text{C}]$ceftiofur and unlabelled drug. In some cases, a comparison could be made between total residues, as determined by the total radioactivity recovered, and potential antimicrobial residues, determined as desfuroylceftiofur.

The metabolism of ceftiofur has been shown to be more complex than that of other cefalosporins, primarily as a result of the cleavage of its thiol ester bond, which yields desfuroyloceftiofur and furoic acid. The metabolism of ceftiofur by the S-9 microsomal fraction from liver and kidney tissues of various species (rats, pigs, cattle and chickens) has been studied. Ceftiofur was converted in vitro into desfuroyloceftiofur, the primary metabolite in kidney being desfuroyloceftiofur cysteine disulfide. In liver, the primary metabolites were desfuroylceftiofur and 3,3'-desfuroylceftiofur disulfide dimer. Qualitatively, the results observed were the same for all species, but metabolism was slower in S-9 fractions from liver and kidney tissues of rats as compared with S-9 fractions from liver and kidney tissues of the other species tested. The highest concentrations of 3,3'-desfuroylceftiofur disulfide dimer were observed in chicken liver and kidney fractions, while the lowest were found in rat tissue fractions.

In cattle given ceftiofur by intramuscular injection, the metabolism observed was similar to that seen in rats given a single oral dose of 200 mg per kg of body weight or an oral dose of 800 mg per kg of body weight, repeated on 5 successive days, of $[^{14}\text{C}]$ceftiofur. In subsequent studies, the major urinary metabolite in cattle was identified as 3,3'-desfuroyloceftiofur disulfide dimer. The active metabolite present in plasma in all species studied was desfuroylceftiofur, and this was also the first compound formed in the metabolic pathway in tissues. About 95% of ceftiofur residues were excreted in urine and faeces within 24 hours.

Pharmacokinetic studies in calves, beef cattle and dairy cattle showed that the depletion curve of total radiolabelled residues of ceftiofur in cattle was biphasic. The tissue half-life of the initial phase is 1-2 days, and that of the second phase 20-80 days, depending on the tissue.

Three reports described the pharmacokinetics of ceftiofur in pigs, based on studies conducted with $[^{14}\text{C}]$ceftiofur. The metabolic profile of ceftiofur in the urine and kidney of pigs treated with $[^{14}\text{C}]$ceftiofur by intramuscular injection matched that previously observed for rats which received the drug by oral administration, as well as for cattle treated by intramuscular injection. In pig kidney, 37% of the total residues were free, desfuroylceftiofur cysteine disulfide being the principal free metabolite. As observed in other species, desfuroylceftiofur was the major extractable metabolite, which has a half-life in blood of 12-15 hours.
Five residue-depletion studies in cattle treated with \(^{14}\text{C}\)ceftiofur were considered, together with two residue-depletion studies in calves given the unlabelled drug, where residues expressed as desfuroyloceftiofur were reported. In studies in cattle and calves treated with five successive daily intramuscular injections of 2.2 mg per kg of body weight \(^{14}\text{C}\)ceftiofur, the highest residues in kidney (5.5 mg/kg) were seen when animals were killed at 8 hours after dosing; total residues in muscle were 0.23 mg/kg. Kidneys from cattle slaughtered at 5 days withdrawal following five daily treatments with 2.3 mg per kg of body weight \(^{14}\text{C}\)ceftiofur contained 2.5 mg/kg total ceftiofur residues, while liver contained 0.37 mg/kg and fat and muscle <0.1 mg/kg. In calves treated with three successive daily intramuscular injections of 2.2 mg per kg of body weight \(^{14}\text{C}\)ceftiofur, residue concentrations were <1.0 mg/kg in all tissues at 3 days withdrawal; concentrations in muscle were 0.02 mg/kg. In the typical distribution of desfuroyloceftiofur residues in edible tissues of calves treated with a single intramuscular dose of 1 mg per kg of body weight ceftiofur, residues in liver amounted to 1.42 mg/kg at 2.5 hours, declining to 1.05 mg/kg at 7.5 hours. Residues in kidney and injection sites declined from 1.28 and 1.86 mg/kg to 1.20 and 0.60 mg/kg, respectively, over the same period. Other experiments in calves given five daily intramuscular injections of 1.1 mg per kg of body weight of ceftiofur free acid equivalents (CFAE) demonstrated that desfuroyloceftiofur residues in calf kidneys decline from 0.62 mg/kg at 24 hours after dosing to 0.14 mg/kg at 48 hours, and that tissues were generally free of desfuroyloceftiofur residues at longer withdrawal times.

The distribution of desfuroyloceftiofur residues in the edible tissues of pigs killed 12 hours after the third of three daily intramuscular injections of 3 mg of unlabelled CFAE was as follows: kidney, 2.17 mg/kg; liver, 1.51 mg/kg; fat, 0.40 mg/kg; injection site muscle, 0.19 mg/kg; muscle, <0.2 mg/kg. At 2 days, there were no detectable residues, except in the samples of fatty tissue (bacon), which contained an average of 0.19 mg/kg desfuroyloceftiofur, and in one of five liver samples, which contained 0.21 mg/kg. In pigs slaughtered at 12 hours after the third of three daily intramuscular injections of 5.2 mg per kg of body weight \(^{14}\text{C}\)ceftiofur, the concentration of total residues in kidney was 4.47 mg/kg, while concentrations in muscle, liver and fat were 0.76, 1.55 and 1.49 mg/kg, respectively.

Four studies were considered in which lactating dairy cattle were treated with radiolabelled or unlabelled ceftiofur. In the largest of these studies, 48 lactating Holstein dairy cows each received 2.2 mg of unlabelled ceftiofur per kg of body weight by intramuscular injection on 5 successive days. Milk was collected from the various groups at 1, 2, 4, 6, 8, 10, 12 or 14 hours after the first and last injections, and samples were analysed by HPLC. Residues of desfuroyloceftiofur peaked at 72 mg/l at 10 hours after treatment and were <60 mg/l at all other sampling times. In studies with \(^{14}\text{C}\)ceftiofur, maximum residue levels of 111 mg/l, based
on total radioactivity recovered, were found at 12 hours after dosing. Total residues were similar at 12 hours after each of a series of five injections at 24-hour intervals.

**Analytical method**

The HPLC method for the determination of desfuroylceftiofur in various matrices, including bovine plasma, liver, kidney, fat, muscle and milk, and porcine kidney and muscle, has quantification limits in the range 30–100 μg/kg for tissues and 50 μg/l for fluids, detection limits being about half these values. The method is based on the release of bound residues using dithioerythritol in phosphate buffer, followed by purification on solid-phase extraction cartridges. The desfuroylceftiofur is derivatized to form desfuroylceftiofur acetamide and analysed on an HPLC column under gradient conditions with ultraviolet detection at 254 nm. Recoveries were reported to be in the range 80–100%, while coefficients of variation of 4–17% were reported. The method, which includes some minor variations to allow for differences between the various sample matrices tested, has been used for the analysis of residues in tissues of animals following treatment with ceftiofur as well as in tissues fortified with the drug, but has not undergone interlaboratory validation.

**Maximum Residue Limits**

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

- An ADI of 0–50 μg per kg of body weight was established, based on a microbiological end-point. This would result in a maximum ADI of 3000 μg for a 60-kg person.
- Kidney and muscle were considered to be the appropriate target tissues.
- The product is used in lactating cows.
- A significant proportion of the residue is present as non-extractable protein-bound material that does not include an intact β-lactam structure.
- Desfuroylceftiofur is the appropriate marker residue and accounts for all residues that include an intact β-lactam structure.
- Quantification limits for the HPLC method for desfuroylceftiofur are 50 μg/kg for bovine muscle and kidney, 100 μg/kg for bovine liver and fat, 50 μg/l for cows’ milk, and 30 and 100 μg/kg for porcine muscle and kidney, respectively.

The Committee recommended MRLs for ceftiofur of 200 μg/kg for muscle, 2000 μg/kg for liver, 4000 μg/kg for kidney and 600 μg/kg for fat in both cattle and pigs, as well as 100 μg/l for cows’ milk, expressed as desfuroylceftiofur.
The MRLs recommended above would result in a daily maximum intake of 640 \( \mu \)g of ceftiofur residues, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, and 1.5 l of milk (Annex 1, reference 85).

3.2.2 **Chlortetracycline and tetracycline**

Chlortetracycline and tetracycline are broad-spectrum antimicrobial drugs with a long history of use in humans and animals. Tetracycline is used primarily for the short-term oral treatment of clinical diseases, while chlortetracycline is usually administered in the feed or water for prophylactic purposes.

The tetracyclines as a group were previously evaluated at the twelfth meeting of the Committee (Annex 1, reference 17), when a temporary ADI of 0–0.15 mg per kg of body weight was established. Oxytetracycline was re-evaluated at the thirty-sixth meeting of the Committee (Annex 1, reference 91), when an ADI of 0–3 \( \mu \)g per kg of body weight was established, based on effects on the human gut flora. Additional data have become available on chlortetracycline and tetracycline since the twelfth meeting, which were evaluated at the present meeting.

Chlortetracycline and tetracycline are closely related in structure. The toxicological profiles and spectra of antimicrobial and biological activity are similar and therefore the Committee considered data on the two compounds together in evaluating their toxicological potential.

**Toxicological data**

The Committee considered data from studies on pharmacokinetics, metabolism, acute and short-term toxicity, reproductive toxicity, teratogenicity, long-term toxicity/carcinogenicity, genotoxicity, antimicrobial effects and observations in humans. Aspects of the toxicity of these compounds as compared with oxytetracycline were also considered.

Pharmacokinetic studies showed that chlortetracycline and tetracycline were rapidly absorbed following oral administration. Peak plasma concentrations were higher in rats receiving tetracycline than in those receiving the same dose of chlortetracycline. In humans receiving oral therapeutic doses, 30% of the dose of chlortetracycline was absorbed, as compared with 60–80% of the tetracycline dose. Tetracycline is excreted mainly in urine, whereas chlortetracycline is excreted in urine and faeces in comparable amounts. After absorption following administration by various routes, chlortetracycline and tetracycline are widely distributed in the body; the highest levels are present in the liver and kidney. Detectable levels of tetracyclines in bone persist for more than 28 weeks after dosing. The plasma half-lives of chlortetracycline and tetracycline in humans are about 5 and 8 hours, respectively. Tetracyclines have been found in the breast milk of women receiving therapeutic doses and can cross the placenta.
Orally administered chlortetracycline and tetracycline have low acute toxicity in rats and mice, LD₅₀s ranging from 2150 to >5000 mg per kg of body weight.

In short-term toxicity studies with mice in which tetracycline was incorporated in the diet at concentrations equivalent to 440, 900, 1800, 3600 or 7150 mg per kg of body weight per day, the only sign of toxicity observed was a decrease in body weight in the highest-dose group. In a 13-week range-finding study in which rats were dosed at 155, 315, 625, 1250 or 2500 mg tetracycline per kg of body weight per day, vacuolization of liver cells and bone marrow atrophy were observed at 1250 and 2500 mg per kg of body weight per day. No toxicity was observed in dogs given tetracycline orally at dose levels of up to 250 mg per kg of body weight per day for 24 months. Chlortetracycline did not cause any toxic effects in short-term toxicity studies with rats or mice at doses of up to 200 mg per kg of body weight per day. In dogs given chlortetracycline orally at a dose of 250 mg per kg of body weight per day for 98 days, increased incidences of mortality, fatty liver and bone marrow atrophy were observed. In another experiment in dogs which received chlortetracycline orally at doses of up to 100 mg per kg of body weight per day for 54 weeks, no effects were observed, except for signs of gastrointestinal disorders; these were considered of minor importance because the increase in body-weight gain was normal.

In carcinogenicity studies with mice and rats, tetracycline was administered in the diet at doses of up to 3500 and 1060 mg per kg of body weight per day, respectively. Survival rates were increased in male mice, while body weights were slightly lower than controls for both sexes. Survival rates were also increased in female rats, but male rats showed a dose-related increase in basophilic cytoplasmic changes and clear cell changes in the liver. There were no increases in tumour incidence in mice or rats.

In a long-term toxicity/carcinogenicity study, chlortetracycline was administered in the diet of rats at doses varying from 0.07 to 5200 mg per kg of body weight per day. In the highest-dose group, gastrointestinal irritation, decreased body-weight gain and a decreased number of white blood cells were observed in both sexes, while testicular atrophy was observed in males. Microscopic changes in the highest-dose group included infiltration of monocytes in the lungs of both sexes and fatty changes in the liver in males. Tumour incidence was not increased. The NOEL was 700 mg per kg of body weight per day. The Committee concluded that there was no evidence that either chlortetracycline or tetracycline is carcinogenic.

In a two-generation reproductive toxicity study in which chlortetracycline was administered to rats in the diet at a dose equivalent to 500 mg per kg of body weight per day, no effects on reproductive performance were observed. Reproductive toxicity data were not available on
tetracycline. In a number of teratogenicity studies with rats, tetracycline and tetracycline hydrochloride caused a reduction in ossification at the highest oral dose tested, 540 or 400 mg per kg of body weight per day, respectively. No irreversible structural changes were found on examination of the skeleton or viscera. In studies with a limited number (five per group) of rats and rabbits, no evidence of teratogenicity was found at intravenous doses of 10 mg of chlortetracycline or tetracycline per kg of body weight per day, administered on days 10–20 of pregnancy. The Committee concluded that chlortetracycline and tetracycline did not cause any significant toxic effects on either reproduction or development.

The genotoxicity of chlortetracycline and tetracycline was investigated in a wide range of in vitro and in vivo test systems. Chlortetracycline gave negative results in all tests. Tetracycline was negative in the majority of in vitro and in vivo assays, but induced gene mutations in one in vitro test in mammalian cells and chromosomal aberrations in plants. The Committee considered these effects to be due to the inhibiting effect of tetracyclines on protein synthesis, based on their binding to ribosomes, and concluded that chlortetracycline and tetracycline do not pose a genotoxic hazard.

**Microbiological data**

In vitro studies of the antimicrobial effect of tetracyclines on pathogenic microorganisms of animal origin indicated that the activities of chlortetracycline and oxytetracycline are not significantly different. MIC values (geometric mean) for chlortetracycline and oxytetracycline were 0.32 and 0.52 µg/ml, respectively.

In a limited number of microorganisms representative of human gut flora, the antimicrobial activity of chlortetracycline was of the same order as that of oxytetracycline.

The antimicrobial activity of tetracycline was compared with that of oxytetracycline using a wide range of bacterial species isolated from the faeces of healthy human volunteers. The sensitivity of most species was similar for tetracycline and oxytetracycline, except for *Bifidobacterium*, *Fusobacterium* and *Eubacterium* species, which were slightly more sensitive to tetracycline, and *Streptococcus* species, which were slightly more sensitive to oxytetracycline. The geometric mean MICs for all tested strains were 3.2 µg/ml for tetracycline and 3.8 µg/ml for oxytetracycline. After reviewing the available toxicological and antimicrobial data, the Committee concluded that the antimicrobial data provided the most appropriate end-point for the evaluation of chlortetracycline and tetracycline.

In view of the similarity of the antimicrobial activity of tetracycline, chlortetracycline and oxytetracycline, the Committee established a group ADI of 0–3 µg per kg of body weight for the three drugs, separately or in combination. This ADI was established for oxytetracycline at the thirty-
sixth meeting of the Committee (Annex 1, reference 91), and is based on the NOEL of 2 mg per person per day for the effects of oxytetracycline on the gut flora in human volunteers and a safety factor of 10.

The Committee noted that this ADI provides an adequate margin of safety when compared with the lowest NOEL for toxicological effects of 100 mg per kg of body weight per day for chlortetracycline in dogs.

Residue data
MRLs for chlortetracycline, oxytetracycline and tetracycline in meat, milk and eggs were recommended at the twelfth meeting of the Committee (Annex 1, reference 17). At the thirty-sixth meeting (Annex 1, reference 91), oxytetracycline was re-evaluated. MRLs were established for muscle (100 μg/kg), liver (300 μg/kg), kidney (600 μg/kg), fat (10 μg/kg) and eggs (200 μg/kg), and the MRL for milk of 100 μg/l recommended at the twelfth meeting was retained.

Metabolism data
Both tetracycline and chlortetracycline are rapidly and moderately well absorbed from the gastrointestinal tract and eliminated in both the urine and faeces, either unchanged or in a microbiologically inactive form.

There was no evidence that either chlortetracycline or tetracycline is significantly metabolized in vivo, although some chemical isomerization of the drug can occur in vitro during isolation. A microbiological assay is therefore a satisfactory method of measuring the depletion of tetracyclines from tissues. Since chlortetracycline and tetracycline undergo minimal metabolism, they are the appropriate marker compounds for the determination of residues in tissues.

Pharmacokinetic data
Chlortetracycline. Chlortetracycline is both rapidly absorbed and quickly cleared from edible tissues following oral administration. Typically, 10–20% of the drug is absorbed, peak serum levels being attained within 1–3 hours after dosing. Oral absorption of chlortetracycline depends on the mode of administration and the formulation. Divalent cations such as calcium or magnesium form complexes with chlortetracycline that reduce the oral bioavailability, and the presence or absence of these cations in animal feed influences drug absorption. The adverse effects of divalent cations on chlortetracycline bioavailability can be minimized by giving a competitive complexing agent, such as citric acid, at the same time. In general, oral administration in drinking-water gives the highest plasma residue concentrations.

Tetracycline. Tetracycline is both rapidly absorbed and quickly cleared from edible tissues following oral administration. Typically, 10–20% of the drug is absorbed, peak serum levels being attained within 2 hours after oral dosing. Like chlortetracycline, the oral absorption of tetracycline is decreased by the presence of divalent cations.
Residue data

Chlortetracycline. In pigs receiving prophylactic chlortetracycline at a dose rate of 400 mg/kg in the diet for 7 days, mean residue levels were 0.15 and 0.11 mg/kg in kidney and 0.11 and 0.08 mg/kg in liver, 3 and 5 days, respectively, after withdrawal of medication. Administration of 198 mg/kg of chlortetracycline in drinking-water for 5 days resulted in residue levels of 0.31 and 0.05 mg/kg in kidney and liver respectively, 2 days after withdrawal of the drug.

In calves given feed containing 20 mg/kg of chlortetracycline for 7 days, mean residue levels were 0.12 and 0.04 mg/kg in kidney and liver, respectively, 15 days after withdrawal of medication. Dosing of calves with 22 mg/kg of chlortetracycline, either as a soluble bolus or in drinking-water, for 2-10 days gave residue levels of 0.45 and 0.27 mg/kg in kidney and liver, respectively, 7 days after drug withdrawal.

Cattle given feed containing 22 mg/kg of chlortetracycline had residue levels in kidney and liver of 0.20 and 0.10 mg/kg, respectively, 5 days after withdrawal of medication. Lactating cows given a single intrauterine dose of 2 g of chlortetracycline (as a soluble bolus) had residue levels in milk of <0.05 mg/l 3 days after dosing. In a lactating cow given a single dose of 3 g by intrauterine infusion, residue levels in milk were <0.15 mg/l 84 hours after dosing. In lactating cows which received chlortetracycline by intramammary infusion at 426 mg per day for 5 days, mean residue levels in milk were 0.07 mg/l 4.5 days after cessation of treatment.

Administration of drinking-water containing 528 mg/l chlortetracycline together with feed containing 200 mg/kg to broiler chickens for 3 days resulted in average residue levels of 0.5 and 0.09 mg/kg in kidney and liver, respectively, 2 days after withdrawal of the drug. Chickens given 200 mg/kg chlortetracycline continuously in the diet had mean residue levels of 0.3 and 0.05 mg/kg in kidney and liver, respectively, 1 day after withdrawal of the drug. Residue levels in eggs were <0.05 mg/kg at the time of withdrawal when the drug was administered at the rate of 120 mg/l in drinking-water for 7 days, but doses of 600 mg/kg in feed over the same period gave residue levels of 0.19 mg/kg 1 day after withdrawal. Turkeys given feed containing 600 mg/kg of chlortetracycline had residue levels averaging 0.4 and 0.1 mg/kg in kidney and liver, respectively, 4 days after withdrawal. In a study in which turkeys were dosed with 528 mg/l of the drug in drinking-water for 3 days, mean residue levels in the kidney and liver were 0.4 and 0.1 mg/kg, respectively, at 4 days after treatment.

Kidney and liver tissues in all species show the highest concentration of chlortetracycline both at the time of withdrawal from medication, and at all times during the withdrawal period. These are also the last tissues to contain detectable chlortetracycline residues. Residue levels in muscle
were less than 10% of those found in kidney and liver during withdrawal from medication, and values in fat were considerably lower than those in muscle.

Two limited residue-depletion studies were reported in lambs fed continuously with a fattening ration containing 50 mg/kg of chlortetracycline. At the time of withdrawal, the kidney, liver, muscle and fat contained 0.33, 0.11, 0.027 and <0.025 mg/kg of chlortetracycline residues, respectively. No residues were detected in these tissues at 4 days withdrawal.

*Tetracycline.* In pigs given tetracycline at 24 mg per kg of body weight per day for 14 days in drinking-water, mean residue levels were 0.2 and 0.1 mg/kg in kidney and liver, respectively, 4 days after withdrawal of medication. Calves on the same dose had average residue levels of 0.4 and 0.17 mg/kg in kidney and liver, respectively, 10 days after withdrawal of medication. In chickens given 620 mg/l of tetracycline in drinking-water for 5 days, average residue levels in kidney and liver were 1.4 and 0.2 mg/kg, respectively, 24 hours after withdrawal of the drug. Residues in whole egg averaged 0.27 mg/kg at 4 days withdrawal, declining to <0.06 mg/kg 10 days after withdrawal. A dose of 3 g given to a lactating cow by intraterine infusion led to residues of <0.10 mg/kg in milk 84 hours after dosing.

**Analytical methods**

Chlortetracycline and tetracycline residues may be measured by either microbiological or chemical assays. Chemical analysis by HPLC with either ultraviolet or fluorescence detection is much more sensitive than microbiological assays, the quantification limit being 20 µg/kg in tissues and 10 µg/kg in milk. However, the facility with which tetracyclines complex with or bind to many different substances demands careful sample preparation under stringently controlled conditions to ensure statistically satisfactory recoveries of target drug. Recoveries of about 60–75% are typical in the analysis of tetracycline residues. Tissue-depletion measurements conducted both by microbiological and chemical assays give very similar results.

**Maximum Residue Limits**

In reaching its decision on MRLs for chlortetracycline and tetracycline, the Committee considered the following:

- MRLs were recommended for oxytetracycline at the thirty-sixth meeting of the Committee for all species of 600 µg/kg in kidney; 300 µg/kg in liver; 100 µg/kg in muscle; 100 µg/kg in milk; 200 µg/kg in eggs; and 10 µg/kg in fat. These levels were the lowest detectable by validated antimicrobial methods.
- Chlortetracycline and tetracycline have been allocated a group ADI of 0-3 µg per kg of body weight with oxytetracycline.
• Modern analytical techniques allow much more sensitive and specific assays than those provided by antimicrobial inhibition assays.
• The recommended target tissues for residue analysis in cattle, pigs and poultry are kidney and muscle. Based on limited data, the kidney is the recommended target tissue in sheep.
• The marker residue for all three substances is parent drug.

The Committee recommended the following temporary MRLs for both chlortetracycline and tetracycline in cattle, pigs and poultry, expressed as parent drug:

- Muscle – 100 μg/kg
- Liver – 300 μg/kg
- Kidney – 600 μg/kg
- Eggs (poultry) – 200 μg/kg.

The Committee also recommended temporary MRLs for sheep liver and kidney of 300 μg/kg and 600 μg/kg, respectively, expressed as parent drug.

The Committee allocated the same ADI and MRLs to chlortetracycline and tetracycline as those previously allocated to oxytetracycline at its thirty-sixth meeting for the given tissues and species.

Although the Committee realized that it is unlikely that tetracyclines will be used in combination, the MRLs allocated to tetracyclines were defined as applying both to residues of individual tetracyclines and to the sum of combined tetracycline residues, including chlortetracycline, oxytetracycline and tetracycline.

The following information is required for evaluation in 1996:

1. The results of residue-depletion studies in cattle, sheep, pigs and poultry treated with these substances in accordance with approved uses, to determine the depletion of residues in milk (cows), fat (all species) and muscle, liver and kidney (sheep).

2. New and validated methods of analysis for chlortetracycline, oxytetracycline and tetracycline residues in tissues and milk.

3.2.3 **Oxytetracycline**

Oxytetracycline was last evaluated at the thirty-sixth meeting of the Committee (Annex 1, reference 97). At that time, MRLs of 100 μg/kg for muscle, 300 μg/kg for liver, 600 μg/kg for kidney, 10 μg/kg for fat and 200 μg/kg for eggs were recommended. The MRL of 100 μg/kg for milk recommended at the twelfth meeting (Annex 1, reference 17) was retained. The Codex Alimentarius Commission has adopted these MRLs for the species in which residues were tested, namely cattle, sheep, pigs, turkeys and chickens (muscle, liver, kidney and fat), cattle (milk) and
chickens (eggs). At its present meeting, the Committee was requested to review data relating to residues in the giant tiger prawn (*Penaeus monodon*).

An ADI of 0–3 µg per kg of body weight was established for oxytetracycline at the thirty-sixth meeting of the Committee (Annex 1, reference 91). This was converted into a group ADI for this drug together with chlorotetracycline and tetracycline at the present meeting.

Oxytetracycline is a broad-spectrum antibiotic that is used for the treatment and control of a variety of Gram-negative bacterial infections in humans and animals, including vibriosis caused by bacteria in giant tiger prawns. It is generally given to prawns mixed with prepared diets, often as a formulated medicated diet at a dose range of 2–5 g/kg ad lib for 5 days.

**Metabolism data**

Studies on the metabolism of oxytetracycline in humans, dogs and rats were evaluated by the Committee at its thirty-sixth meeting (Annex 1, reference 91), and it was concluded that the drug is unlikely to be metabolized in these species.

**Pharmacokinetic data**

The absorption, clearance, tissue distribution and excretion of oxytetracycline were studied in giant tiger prawns, which received the drug orally at dose levels of 11 and 22 mg per kg of body weight, and were then maintained at 28–30 °C. During this study, 10 prawns, each weighing 30–40 g, were collected randomly at withdrawal times ranging from 0.5 hours to 10 days. Oxytetracycline was shown to be poorly absorbed, reaching peak concentrations in the tissues 8 hours after dosing.

**Residue data**

Residue studies were carried out in prawns receiving 2.5 g/kg or 5.0 g/kg medicated diet. Pellet or fish flesh diets were given ad lib for 5 days. Six prawns were collected twice daily for 20 days and analysed by an HPLC method with a detection limit of 0.01 mg/kg. Oxytetracycline concentrations were found to be 3–17 mg/kg and 12–40 mg/kg in muscle tissues of prawns receiving 2.5 g/kg and 5 g/kg medicated fish flesh diets for 5 days, respectively, as compared with 0.2–1.5 mg/kg and 1–3 mg/kg, respectively, when the medicated pellet diets were used. The mean maximum residue concentrations observed at 1 day after withdrawal of the 2.5 g/kg diet were 1.2 and 0.45 mg/kg for the medicated fish flesh and pellet diets, respectively. The corresponding figures for the residues for the 5 g/kg diet were 20.0 and 0.75 mg/kg.

Residues in muscle tissue were detected up to 11 days and 3 days after withdrawal of the medicated fish flesh and pellet diets, respectively. The half-life of oxytetracycline in prawns given the medicated fish flesh diet was 1.2 days.
Exposure from the environment

The Committee noted that prawns may be subjected to repeated exposure to oxytetracycline since sediments may be contaminated by medicated feed or by faeces containing the drug. A significant proportion of the drug is, however, in a biologically inactive form, possibly as a result of the formation of complexes of oxytetracycline with divalent cations present in the aquatic environment. Therefore, exposure from sediment was not considered to be of major concern.

Analytical methods

Modified HPLC methods were used in the prawn studies. In one method, oxytetracycline was extracted using oxalic acid and edetic acid (ethylenediaminetetraacetic acid). With this modification, a recovery of 75% was attained with a detection limit of 0.01 mg/kg. Another modified HPLC method based on metal chelate affinity chromatography was reported to have a quantification limit of 0.01 mg/kg and 74% recovery.

Microbiological assays were not recommended because the detection limit in prawns of 1 mg/kg is not adequate for residue analysis.

Maximum Residue Limits

An MRL of 100 µg/kg was recommended for muscle in all species at the thirty-sixth meeting of the Committee (Annex 1, reference 91). At its forty-third meeting (Annex 1, reference 113), the Committee considered the human consumption of farmed prawns and adopted a daily intake value of 300 g of muscle and skin in natural proportions. The Committee recommended this value as an alternative to the meat consumption factor that is normally used.

At its present meeting, the Committee recommended a temporary MRL for oxytetracycline of 100 µg/kg for the edible tissue of Penaeus monodon, expressed as parent drug. The MRL is temporary pending the availability of a validated analytical method. Such a method is required for review in 1996.

3.3 Antiprotozoal agent

3.3.1 Diclazuril

Diclazuril had not previously been reviewed by the Committee.

Diclazuril is an anticoccidial drug used in major poultry species, such as broiler chickens, pullets and turkeys, as well as in rabbits and lambs.

Toxicological data

The Committee considered data from studies on acute and short-term toxicity, long-term toxicity/carcinogenicity, reproductive and developmental toxicity, metabolism, pharmacokinetics and antimicrobial effects.
Pharmacokinetic studies in rats given radiolabelled drug suggested that the absorption of diclazuril from the gut was limited, as 90% of the radioactive dose was excreted in the faeces within 24 hours. After 4 days, 92% of the dose had been excreted in the faeces and 0.04% in the urine. Unchanged diclazuril accounted for most of the total radioactivity in the faeces, with two metabolites accounting for less than 0.5%. Distribution into the tissues was rapid but limited. The concentration of total radioactivity in the liver was about half that in the plasma; the corresponding figures for kidney, lung and heart were in the range 20–30%. With time, metabolites gradually accounted for a higher proportion of the tissue radioactivity.

Single oral doses of diclazuril of up to 5 g per kg of body weight caused no mortality in experimental animals. Clinical effects in mice and rats were non-specific and mainly of central nervous system origin. In dogs, vomiting and defecation were seen after treatment.

In a 3-month dose-ranging study in mice, mortality was not seen with oral doses of up to 1600 mg per kg of body weight, equivalent to 240 mg per kg of body weight per day. At this dose, changes indicative of mild liver damage were reported, including an increased relative liver weight and swelling and vacuolization of the centrilobular hepatocytes in males; females showed swelling of the centrilobular hepatocytes only. The NOEL was 30 mg per kg of body weight per day.

In a 3-month toxicity study in mice in which the highest dose was 3000 mg/kg in the diet, equal to 850 mg per kg of body weight per day in males and 920 mg per kg of body weight per day in females, neither mortality nor adverse clinical effects were noted. No mortality occurred in treated mice, but at all dose levels (290–850 mg per kg of body weight per day) males showed decreased serum levels of total bilirubin. This and other haematological and biochemical changes in the blood, although occasionally statistically significant, were either marginal with respect to historical controls or not dose-related. Liver weights were increased at all doses in males and at the highest dose in females. Swelling of the centrilobular hepatocytes was observed in males dosed at 500 and 850 mg per kg of body weight per day. A NOEL was not identified in this study. It was noted that wastage of feed had probably caused overestimation of dietary intake of the drug and that the calculated dosage was about twice that derived from dietary intake conversion tables.

Rats were given diclazuril at doses of 50–800 mg/kg in the diet, equal to 4–69 mg per kg of body weight per day in males and 6–89 mg per kg of body weight per day in females, for 3 months. Male rats dosed at 17 and 69 mg per kg of body weight per day showed swelling and vacuolization of the centrilobular hepatocytes. Absolute and relative liver weights were increased in both males and females at the highest dose. These changes
fell within the range of historical controls. The NOELs in this study were 4 and 21 mg per kg of body weight per day for males and females, respectively.

In a second 3-month study, in which rats were dosed at 1000-3000 mg/kg in the diet, equal to 71-210 mg per kg of body weight per day in males and 82-240 mg per kg of body weight per day in females, swelling of the centrilobular hepatocytes was seen in both sexes at all doses. Vacuolization of the hepatocytes was noted in males dosed at 140 mg per kg of body weight per day and above. No deaths occurred in either sex at any dose level in this study, nor was any relevant change revealed by biochemical analyses, haematology or urinalysis. Relative liver weights were, however, increased in males and females in the highest-dose group. A NOEL was not identified in this study.

In a 12-month study in rats given dicycluril at doses of 16-1000 mg/kg in the diet, equal to 1-74 mg per kg of body weight per day in males and 2-88 mg per kg of body weight per day in females, no drug-related changes were observed at doses of up to 6 or 18 mg per kg of body weight per day for females or males, respectively. Histological examination showed aggregates of histiocytes in the mesenteric lymph node in males at 74 mg per kg of body weight per day and at both 23 and 88 mg per kg of body weight per day in females. Swelling of centrilobular hepatocytes was seen in males dosed at 74 mg per kg of body weight per day, while females dosed at 88 mg per kg of body weight per day had increased clusters of foamy cells in the lungs. The NOEL was 6 mg per kg of body weight per day for both sexes.

Dicycluril capsules were administered to dogs (four males and four females per group) at doses of 5, 20 or 80 mg per kg of body weight per day for 3 months. Two additional male and female animals were included in the control and highest-dose groups in a 1-month withdrawal study. At 80 mg per kg of body weight per day in both males and females, a fine granular, yellow-brown pigment was present in the cytoplasm of hepatocytes. In males at this dose level, a significant increase in serum urea (expressed as nitrogen) was also seen. Both changes were reversible, as shown by the four animals of the recovery group. A 12-month toxicity study in dogs of the same design and dose levels as the 3-month study but lacking a recovery group, confirmed the findings of the earlier study. The liver changes were comparable in nature and intensity in both studies. The NOEL in these studies was 20 mg per kg of body weight per day.

A 25-month combined long-term toxicity/carcinogenicity study was performed in mice. This study included periodic haematological examinations, analysis of serum samples at the end of the study and extensive histopathological examinations. Dicycluril was administered at dose levels of 16, 63, 250 or 1000 mg/kg in the diet, equal to 3, 11, 47 or 190 mg per kg of body weight per day in males and 4, 14, 53 or 220 mg per kg of body weight per day in females. While there was evidence of
inhibition of the rate of body-weight gain in male mice at the highest dose, histopathological examination revealed only minor hepatic morphological changes of the type also found in the 3-month studies in mice. In view of the results of the two 3-month studies in mice of the same strain, the Committee considered that the toxic potential of doses exceeding those used in the carcinogenicity study had been evaluated. Analysis of serum samples collected at the end of these two 3-month studies provided evidence of the existence of a threshold for the absorption of diclazuril of between 1000 and 2000 mg/kg in the diet. This threshold is close to the highest dose used in the long-term toxicity/carcinogenicity study. In the light of this finding, together with the absence of any biologically significant increase in the incidence of tumours, the Committee concluded that diclazuril had been adequately tested and was not carcinogenic in mice. The NOEL in this study was 3 mg per kg of body weight per day.

A 28-month combined long-term toxicity/carcinogenicity study was performed in rats given 16, 63, 250 or 1000 mg/kg diclazuril in the feed (equal to 1, 4, 15 or 61 mg per kg of body weight per day in males and 1, 5, 20 or 80 mg per kg of body weight per day in females). This study included periodic haematological examinations, analysis of serum samples at the end of the study and extensive histopathological examinations. As in the study with mice, no effects of obvious toxicological significance were observed, but reactive histiocytosis of the mesenteric lymph nodes was present in female rats at 20 and 80 mg per kg of body weight per day and in males at 61 mg per kg of body weight per day. However, as with the studies in mice, serum samples collected at the end of the two 3-month studies allowed an exploration of the relationship between the oral intake of diclazuril and the resultant concentration in serum. A threshold existed for the absorption of diclazuril in the region of 2000 mg/kg in the feed, i.e. at approximately twice the highest dose used in the long-term toxicity/carcinogenicity study. In the light of this finding, together with the absence of any biologically significant increase in the incidence of tumours, the Committee concluded that diclazuril had been adequately tested and was not carcinogenic in the rat. The NOEL in this study was 4 mg per kg of body weight per day.

A two-generation reproductive toxicity study was performed in rats, in which each generation produced two litters. The doses were equivalent to 5, 20 or 80 mg per kg of body weight per day. In the first generation, the only adverse effect observed was a reduction in birth weight of pups at 80 mg per kg of body weight per day. In the second generation, body-weight gain of dams during pregnancy was slightly lower at 80 mg per kg of body weight per day, and food consumption during pregnancy and lactation was decreased at 20 and 80 mg per kg of body weight per day, indicating maternal toxicity at these doses. As in the first generation, birth weights were lower at 80 mg per kg of body weight per day, and the
survival rates and weight of pups at weaning were also decreased at 20 and 80 mg per kg of body weight per day. The NOEL in this study was 5 mg per kg of body weight per day.

In two teratogenicity studies in rats, diclazuril was administered in the feed at doses equivalent to 1–160 mg per kg of body weight per day from days 6 to 16 of pregnancy. Dosing equivalent to 1 and 5 mg per kg of body weight per day did not result in any adverse effects on the dams or their progeny. At a dose equivalent to 20 mg per kg of body weight per day, however, slight maternal toxicity was observed, characterized by a reduction in body-weight gain. Litter weights were decreased in groups receiving 20–160 mg per kg of body weight per day. At none of the doses was teratogenicity evident. The NOEL in these studies was 5 mg per kg of body weight per day.

In two studies in rabbits, administration of diclazuril by gavage at doses of 5–160 mg per kg of body weight per day from days 6 to 18 of pregnancy did not result in any adverse effects on the dams or their offspring. From these findings and because studies of the enteric absorption of diclazuril in the rabbit were lacking, the Committee concluded that there was no evidence that exposure to diclazuril had been sufficient to enable the teratogenicity of the drug to be evaluated. This conclusion was supported by the occurrence of adverse effects in rabbits exposed to Eimeria species following administration of diclazuril in the feed at a dose equivalent to 13 mg per kg of body weight per day for 5 weeks. Furthermore, the Committee noted that maternal toxicity and fetotoxicity had been observed in rabbits dosed with a diclazuril analogue at 80 mg per kg of body weight per day.

Diclazuril was studied in a range of in vivo and in vitro assays with a variety of genetic end-points. Negative findings in all these assays enabled the Committee to conclude that diclazuril was not genotoxic.

The Committee established a temporary ADI of 0–20 µg per kg of body weight for diclazuril, based on the NOEL of 3 mg per kg of body weight per day in the 2-year toxicity/carcinogenicity study in mice and a safety factor of 200. The ADI was rounded to one significant figure, consistent with accepted rounding procedures (Annex 1, reference 91, section 2.7).

The results of a teratogenicity study in rabbits, supported by evidence that the doses administered were sufficiently high for the teratogenic potential of diclazuril to be adequately explored, are required for evaluation in 1998.

**Pharmacokinetic data**

Extensive pharmacokinetic studies were conducted using [14C]diclazuril in laboratory animals and food animal and bird species. In rats, the depletion of radiolabelled drug was monophasic, the half-life in plasma being 53 hours for total residues and 36 hours for parent drug. Distribution to systemic tissues was rapid but limited. Maximum plasma
concentrations of total residues and unchanged parent drug were similar, approximately 1 mg/l at 8 hours after dosing. More than 90% of the dose was excreted in the faeces within 24 hours and about 97% within 96 hours. Several minor metabolites were detected in faeces; however, the most abundant metabolite accounted for less than 1% of the radiolabel, indicating that metabolism was very limited in this species. Pharmacokinetic studies failed to reveal any bound residues. Residue levels were highest in the liver, followed by the kidney, lung, heart, muscle and brain. Autoradiography data were consistent with residue data.

Similar results were obtained in pharmacokinetic studies in food-producing animals. In rabbits given a single oral dose of 1 mg of [14C]diclazuril per kg of body weight in a gelatin capsule, more than 70% was recovered in faeces and 3% in urine within 48 hours. The two main metabolites were a glucuronide and a sulfate conjugate. None of the urinary or faecal metabolites accounted for more than 2% of the dose, and more than 98% of the dose was excreted within 10 days. Parent drug accounted for almost all the radioactivity in plasma samples collected up to 120 hours after dosing. The distribution of residues was limited, with the liver containing the highest concentrations. The half-life of radioactivity in liver was approximately 3 days and 89–96% was extractable. Residue levels in muscle tissue did not exceed 0.01 mg/kg, calculated as parent drug equivalents. In rabbits which received unlabelled diclazuril in the feed at a dose equivalent to 0.067 mg per kg of body weight per day for 14 days, maximum concentrations of 0.9–1.0 mg/l (calculated as parent drug equivalents) in plasma were reached within 10 days. The residue levels in plasma were maintained for at least 24 hours after withdrawal of the medicated feed, which was attributed to a re-uptake of residues from the faeces.

In broilers which received a single oral dose of 1 mg of [14C]diclazuril per kg of body weight (in a gelatin capsule), maximum concentrations of 1.5–2.0 mg/l (calculated as parent drug equivalents) in plasma were reached at 6 hours after dosing. The elimination half-life of diclazuril was approximately 50 hours in plasma and tissues. Equilibrium rapidly occurred between plasma and tissues; the concentration of residues in the tissues was lower than that in plasma by a factor of 2–10. Residue concentrations were highest in the liver, reaching a maximum of 1.26 mg/kg (calculated as parent drug equivalents) 6 hours after dosing. The corresponding concentrations in kidney, muscle and skin/fat were 1.07, 0.17 and 0.14 mg/kg respectively. Parent drug accounted for more than 90% of the radioactivity in liver samples collected 24 hours after dosing. In broilers which received [14C]diclazuril orally in a gelatin capsule at a dose level of 0.045 mg per kg of body weight twice daily for 14 days, total radioactivity depleted at similar rates from plasma and tissues; the half-life was 2.5 days. Under steady state conditions, about 90% of the daily dose was excreted per day.

Similar studies were performed with [14C]diclazuril in turkeys, using single and multiple oral dosing regimens. The dose levels used were 1 mg
per kg of body weight (single-dose regimen) or 0.025 mg per kg of body weight twice daily for 14 days. Residue concentrations, as determined by radioactivity, were comparable to those obtained in broilers. The elimination half-life of residues was 38 hours in plasma. The half-life of the radioactivity in muscle, kidney and skin/fat was 34–37 hours, as compared with 46 hours in liver. As with broilers, plasma radioactivity could be completely recovered in deproteinized supernatants, and was identified almost exclusively as unchanged drug for up to 1 week after dosing. Tissue concentrations were lower than the corresponding plasma concentrations, liver and kidney showing the highest tissue concentrations. Parent drug accounted for 98% of the radioactivity in liver samples at 6 hours after dosing, and for 85% after 48 and 72 hours. No single metabolite accounted for more than 10% of the radioactivity in liver.

Limited absorption occurred in adult sheep after oral administration at 1 mg per kg of body weight of a 0.25% suspension. Maximum residue concentrations in plasma were 0.012–0.016 mg/l at 24 and 48 hours after dosing. In a second study, in which 4-week-old lambs received two oral doses of 1 mg per kg of body weight of a 0.25% suspension, given 3 weeks apart, bioavailability appeared to be higher. Maximum plasma concentrations occurred 24 hours after dosing and were 0.15 mg/l after the first dose and 0.08 mg/l after the second. No metabolism studies in sheep using radiolabelled drug were reported. However, the results of comparative in vitro studies using ovine hepatocytes indicated that the metabolism of diclazuril in sheep was similar to that in other species.

Residue data
Residue-depletion studies were reported in rabbits, broilers, turkeys and lambs. Rabbits were treated with diclazuril at 1 mg/kg in the feed for 2 weeks, and edible tissues were sampled at 24 hours and 7 days after dosing. Residue levels were determined by HPLC and were highest in liver (1.59 and 0.71 mg/kg at 24 hours and 7 days, respectively), followed by kidney (0.64 and <0.16 mg/kg, respectively) and fat (<0.21 and <0.1 mg/kg, respectively). Residue levels in muscle were below the limit of quantification (0.1 mg/kg) at both sampling times.

Residue studies in broilers were reported in which birds received 1 mg/kg diclazuril in the feed for a complete growing cycle. In a 46-day study using feed derived from a medicated premix containing 0.5% diclazuril at a final concentration of 1 mg/kg in the feed, residue levels at 6 hours after dosing were 0.09, 0.42 and 0.52 mg/kg in muscle, liver and kidney respectively, and below the limit of detection in skin/fat.

In a comparable study using feed derived from a medicated premix containing 0.2% diclazuril at a final concentration of 1 mg/kg in the feed, residue levels at 6 hours after dosing in muscle, liver, kidney and skin/fat were 0.05, 0.37, 0.31 and 0.14 mg/kg, respectively.
Similar studies were reported for turkeys using a 0.5% premix. Residue levels at 6 hours after dosing in liver, kidney, muscle and skin/fat were 0.57, 0.30, <0.10 and 0.16 mg/kg, respectively. With the 0.2% premix, the corresponding values in liver, kidney, muscle and skin/fat were 0.40, 0.29, 0.05 and 0.15 mg/kg, respectively.

A single- and multiple-dose protocol was used in a residue-depletion study in lambs, which received 1 mg per kg of body weight of diclazuril as a 0.25% suspension. In the single-dose treatment, lambs were dosed at 4 weeks of age and residues determined 24 hours after dosing. Residue levels in muscle, liver, kidney and fat were 0.03, 0.30, 0.09 and 0.08 mg/kg, respectively. In the multiple-dose treatment (one dose at 4 weeks and one at 7 weeks), residues were determined 24 hours after the second treatment. Residue levels in muscle, liver, kidney and fat were 0.01, 0.28, 0.04 and 0.04 mg/kg, respectively. Residues were below the quantification limit (0.01 mg/kg) at day 3 for muscle, day 7 for liver, and day 5 for kidney and fat.

The results of the residue-depletion studies in target species using radiolabelled drug indicate that the parent drug is the appropriate marker residue and that the liver is the target tissue for residue testing in abattoirs. For international trade purposes and convenience of analysis, muscle is the target tissue of preference in all species.

**Analytical methods**

A capillary gas-chromatography method (capillary GC) has been developed for quantifying diclazuril residues in chicken liver. The procedure involves derivatization before chromatography and uses an internal standard to aid in quantification, the quantification limit being 0.02 mg/kg. The method has been reported to be specific for diclazuril and interference from chicken liver has not been observed. The GC-electron capture detection (GC-ECD) method is used for measuring residues in plasma, the edible tissues of broilers, turkeys and lambs, and egg (white and yolk). Tissue sample homogenates are fortified with the same internal standard as that used with the capillary GC method. The GC-ECD method has a quantification limit of 0.01 mg/kg in animal tissues and 0.025 mg/kg in egg whites and egg yolks. Residue levels in plasma and the edible tissues of broilers, turkeys and rabbits may also be measured by an HPLC method; however, this method uses a different internal standard. The method involves ultraviolet detection and has a quantification limit of 0.05 mg/kg in edible tissues of rabbits and poultry. Some interference may occur in fat and skin/fat samples when the HPLC method is used at concentrations <0.5 mg/kg in these tissues.

**Maximum Residue Limits**

In recommending temporary MRLs, the Committee took into account the following factors:
• A temporary ADI of 0–20 µg per kg of body weight was established. This would result in a maximum ADI of 1200 µg for a 60-kg person.
• Dicloazuril is absorbed and rapidly eliminated in all species tested with a half-life of 50 hours or less.
• The metabolism of dicloazuril is limited and metabolites account for less than 10% of all residues in all species tested.
• There is no evidence of bound residues in any species.
• Maximum residue concentrations in poultry tissues and in sheep and rabbits occurred at 6 hours and 24 hours after dosing, respectively.
• Liver tissue has the highest concentration of residues and is the recommended target tissue for residue testing in abattoirs, while muscle is the recommended target tissue for international trade purposes.
• Two regulatory methods are available for residue analysis, the GC-ECD method (quantification limit 0.01 mg/kg) and an HPLC method with ultraviolet detection (quantification limit 0.05 mg/kg).
• The MRLs recommended are temporary because the ADI is temporary; additional residue data or analytical methods are not required, because both were considered to be satisfactory.

On the basis of the maximum observed residues in food animals treated with dicloazuril in accordance with good veterinary practices, the Committee recommended temporary MRLs in sheep and rabbits of 500 µg/kg for muscle, 3000 µg/kg for liver, 2000 µg/kg for kidney and 1000 µg/kg for fat, expressed as parent drug. The Committee also recommended temporary MRLs in poultry of 500 µg/kg for muscle, 3000 µg/kg for liver, 2000 µg/kg for kidney and 1000 µg/kg for skin/fat, expressed as parent drug.

From the above temporary MRLs, the theoretical maximum daily intake of dicloazuril residues is 600 µg, based on a daily food intake of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat (Annex 1, reference 83).

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.

3. The Committee wished to draw the attention of FAO and WHO to the need to consider the possible indirect exposure of humans to veterinary drugs through the environment. While recognizing that the evaluation of this additional potential source of contamination of the diet had implications both in terms of the resources required and for
the MRLs which it sets, the Committee recommended that FAO and WHO should consider how this matter could be investigated.

Acknowledgements

The Committee wishes to acknowledge the valuable contributions made to its meeting by: Dr H. Galal-Gorchov, International Programme on Chemical Safety, WHO, Geneva, Switzerland; and Dr Y. Yamaida, Food Standards Officer, Joint FAO/WHO Food Standards Programme, Food 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).


51


30. Evaluation of certain food additives and the contaminants mercury, lead and


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


75. **Specifications for the identity and purity of certain food additives.** FAO Food and Nutrition Paper, No. 37, 1986.


86. **Toxicological evaluation of certain veterinary drug residues in food.** WHO Food Additives Series, No. 25, 1990.

87. **Residues of some veterinary drugs in animals and foods.** FAO Food and Nutrition Paper, No. 41/2, 1990.


89. **Toxicological evaluation of certain food additives and contaminants.** WHO Food Additives Series, No. 26, 1990.

90. **Specifications for the identity and purity of certain food additives.** FAO Food and Nutrition Paper, No. 49, 1990.


## 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>
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<td><strong>Anthelmintic agents</strong></td>
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<tr>
<td>Abamectin</td>
<td>0–0.2 μg/kg of body weight&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No MRLs recommended&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Doramectin</td>
<td>0–0.5 μg/kg of body weight</td>
<td>Muscle (cattle): 10 μg/kg&lt;sup&gt;c,d&lt;/sup&gt; &lt;br&gt;Liver (cattle): 100 μg/kg&lt;sup&gt;c,d&lt;/sup&gt; &lt;br&gt;Kidney (cattle): 30 μg/kg&lt;sup&gt;e,f&lt;/sup&gt; &lt;br&gt;Fat (cattle): 150 μg/kg&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moxidectin</td>
<td>0–2 μg/kg of body weight</td>
<td>Muscle (cattle, sheep and deer&lt;sup&gt;g–i&lt;/sup&gt;): 20 μg/kg&lt;sup&gt;j&lt;/sup&gt; &lt;br&gt;Liver (cattle, sheep and deer&lt;sup&gt;g–i&lt;/sup&gt;): 100 μg/kg&lt;sup&gt;j&lt;/sup&gt; &lt;br&gt;Kidney (cattle, sheep and deer&lt;sup&gt;g–i&lt;/sup&gt;): 50 μg/kg&lt;sup&gt;j&lt;/sup&gt; &lt;br&gt;Fat (cattle, sheep and deer&lt;sup&gt;g–i&lt;/sup&gt;): 500 μg/kg&lt;sup&gt;j&lt;/sup&gt;</td>
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<tr>
<td>Febantel, fenbendazole and oxendazole</td>
<td>0–4 μg/kg of body weight&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Muscle, kidney and fat (cattle, pigs and sheep): 100 μg/kg&lt;sup&gt;h&lt;/sup&gt; &lt;br&gt;Liver (cattle, pigs and sheep): 500 μg/kg&lt;sup&gt;h&lt;/sup&gt; &lt;br&gt;Milk (cattle and sheep): 100 μg&lt;sup&gt;h&lt;/sup&gt;</td>
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<tr>
<td><strong>Antimicrobial agents</strong></td>
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<tr>
<td>Ceftriaxone</td>
<td>0–50 μg/kg of body weight</td>
<td>Muscle (cattle and pigs): 200 μg/kg&lt;sup&gt;i&lt;/sup&gt; &lt;br&gt;Liver (cattle and pigs): 2000 μg/kg&lt;sup&gt;i&lt;/sup&gt; &lt;br&gt;Kidney (cattle and pigs): 4000 μg/kg&lt;sup&gt;j&lt;/sup&gt; &lt;br&gt;Fat (cattle and pigs): 600 μg/kg&lt;sup&gt;j&lt;/sup&gt; &lt;br&gt;Milk (cattle): 100 μg/l&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chlorotetracycline and tetracycline</td>
<td>0–3 μg/kg of body weight&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Muscle (cattle, pigs and poultry): 100 μg/kg&lt;sup&gt;a&lt;/sup&gt; &lt;br&gt;Liver (cattle, pigs, sheep and poultry): 300 μg/kg&lt;sup&gt;a&lt;/sup&gt; &lt;br&gt;Kidney (cattle, pigs, sheep and poultry): 600 μg/kg&lt;sup&gt;a&lt;/sup&gt; &lt;br&gt;Eggs (poultry): 200 μg/kg&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0–3 μg/kg of body weight&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Edible tissue (Penaeus monodon): 100 μg/kg&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Substance</td>
<td>Acceptable Daily Intake (ADI) and other toxicological recommendations</td>
<td>Recommended Maximum Residue Limit (MRL)</td>
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<tr>
<td><em>Antiprotozoal agent</em></td>
<td></td>
<td>Muscle (sheep, rabbits and poultry): 500 µg/kg&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Didazuril</td>
<td>0–20 µg/kg of body weight&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Liver (sheep, rabbits and poultry): 3000 µg/kg&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>Kidney (sheep, rabbits and poultry): 2000 µg/kg&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>Fat (sheep and rabbits): 1000 µg/kg&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>Skin/fat (poultry): 1000 µg/kg&lt;sup&gt;f&lt;/sup&gt;</td>
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**Notes to Annex 2**


* Several issues relating to differences in the approaches adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and JMPR in recommending MRLs in animal products arose at the meeting. In addition, the ADI established by JMPR for abamectin incorporated a safety factor of 500 to take into account the teratogenicity of the Δ-8,9 isomer, which does not occur when abamectin is used as a veterinary drug. For this reason, the Committee did not recommend MRLs for abamectin used as a veterinary drug but recommended that consultations should be held between representatives of JECFA and JMPR to resolve these differences.

* Expressed as parent drug.

* The Committee noted the high concentration of residues at the injection site over a 35-day period after subcutaneous or intramuscular administration of the drug at the recommended dose.

* Temporary MRL (see Annex 3).

* The Committee noted the very high concentrations and great variation in the level of residues at the injection site in cattle over a 49-day period after dosing.

* Group temporary ADI for fentanbacin, fenbendazole and oxfendazole, based on the NOEL for oxfendazole identified at the thirty-eighth meeting of the Committee (WHO Technical Report Series, No. 815, 1991; see Annex 3).

* Determined as the sum of fenbendazole, oxfendazole and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents.

* Expressed as desfuroylceftiofur.

* Group ADI for chlorotetacycline, oxytetracycline and tetracycline, based on the NOEL for oxytetracycline identified at the thirty-sixth meeting of the Committee (WHO Technical Report Series, No. 769, 1993).

* Temporary ADI (see Annex 3).

* The MRL is temporary because the ADI is temporary.
Annex 3

Further toxicological studies and other information required

Anthelminthic agents

*Moxidectin*

Further information on the marker residue in the edible tissues of deer is required for evaluation in 1998.

*Febantel, fenbendazole and oxfendazole*

The following information is required for evaluation in 1998:

- the results of a teratogenicity study in rabbits in which oxfendazole is administered at sufficiently high doses for its teratogenic potential to be adequately explored;
- the results of ongoing residue-depletion studies on total residues of fenbendazole, oxfendazole and oxfendazole sulfone in cattle and sheep following the administration of febantel and oxfendazole.

Antimicrobial agents

*Chlortetracycline and tetracycline*

The following information is required for evaluation in 1996:

- the results of residue-depletion studies in cattle, sheep, pigs and poultry treated with these substances in accordance with approved uses, to determine the depletion of residues in milk (cows), fat (all species) and muscle, liver and kidney (sheep);
- new and validated methods of analysis for chlortetracycline, oxytetracycline and tetracycline residues in tissues and milk.

*Oxytetracycline*

A validated analytical method for the determination of oxytetracycline in *Penaeus monodon* is required for review in 1996.

Antiprotozoal agent

*Diclazuril*

The results of a teratogenicity study in rabbits, supported by evidence that the doses administered were sufficiently high for the teratogenic potential of diclazuril to be adequately explored, are required for evaluation in 1998.
World Health Organization Technical Report Series

Recent reports:
No.        $\text{Sw.fr.}^a$

819 (1992) The hospital in rural and urban districts
Report of a WHO Study Group on the Functions of Hospitals at the First
Referral Level (81 pages) 12.–

820 (1992) Recent advances in medically assisted conception
Report of a WHO Scientific Group (118 pages) 15.–

Fifth report of a WHO Expert Committee on Filariasis (77 pages) 10.–

822 (1992) WHO Expert Committee on Biological Standardization
Forty-second report (89 pages) 12.–

823 (1992) WHO Expert Committee on Specifications for Pharmaceutical
Preparations
Thirty-second report (140 pages) 17.–

824 (1992) WHO Expert Committee on Rabies
Eighth report (90 pages) 12.–

825 (1992) The use of essential drugs
Fifth report of the WHO Expert Committee (79 pages) 10.–

826 (1992) Recent advances in oral health
Report of a WHO Expert Committee (42 pages) 7.–

827 (1992) The role of health centres in the development of urban health systems
Report of a WHO Study Group on Primary Health Care in Urban Areas
(42 pages) 7.–

828 (1992) Evaluation of certain food additives and naturally occurring toxicants
Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food
Additives (57 pages) 9.–

829 (1993) Evaluation of recent changes in the financing of health services
Report of a WHO Study Group (79 pages) 10.–

830 (1993) The control of schistosomiasis
Second report of the WHO Expert Committee (93 pages) 12.–

831 (1993) Rehabilitation after cardiovascular diseases, with special emphasis on
developing countries
Report of a WHO Expert Committee (130 pages) 17.–

832 (1993) Evaluation of certain veterinary drug residues in food
Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives
(68 pages) 10.–

833 (1993) Health promotion in the workplace: alcohol and drug abuse
Report of a WHO Expert Committee (39 pages) 7.–

834 (1993) WHO Expert Committee on Specifications for Pharmaceutical
Preparations
Thirty-third report (35 pages) 7.–

835 (1993) Aging and working capacity
Report of a WHO Study Group (55 pages) 10.–

836 (1993) WHO Expert Committee on Drug Dependence
Twenty-eighth report (50 pages) 10.–

837 (1993) Evaluation of certain food additives and contaminants
Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives
(61 pages) 10.–

838 (1993) Increasing the relevance of education for health professionals
Report of a WHO Study Group on Problem-Solving Education for the Health
Professions (35 pages) 8.–

* Prices in developing countries are 70% of those listed here.

840 (1994) WHO Expert Committee on Biological Standardization
Forty-third report (223 pages)

841 (1994) Cardiovascular disease risk factors: new areas for research
Report of a WHO Scientific Group (59 pages)

842 (1994) Nursing beyond the year 2000
Report of a WHO Study Group (25 pages)

843 (1994) Assessment of fracture risk and its application to screening for postmenopausal osteoporosis
Report of a WHO Study Group (134 pages)

844 (1994) Prevention of diabetes mellitus
Report of a WHO Study Group (108 pages)

845 (1994) Information support for new public health action at district level
Report of a WHO Expert Committee (35 pages)

846 (1994) Fluorides and oral health
Report of a WHO Expert Committee on Oral Health Status and Fluoride Use (42 pages)

847 (1994) Chemotherapy of leprosy
Report of a WHO Study Group (29 pages)

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

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

Sixth report of the WHO Expert Committee (144 pages)

Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives (50 pages)

852 (1995) Onchocerciasis and its control
Report of a WHO Expert Committee on Onchocerciasis Control (111 pages)

853 (1995) Epidemiology and prevention of cardiovascular diseases in elderly people
Report of a WHO Study Group (72 pages)

Report of a WHO Expert Committee (462 pages)

Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives (65 pages)

856 (1995) WHO Expert Committee on Drug Dependence
Twenty-ninth report (21 pages)

857 (1995) Vector control for malaria and other mosquito-borne diseases
Report of a WHO Study Group (97 pages)

858 (1995) WHO Expert Committee on Biological Standardization
Forty-fifth report (108 pages)

859 (1995) Evaluation of certain food additives and contaminants
Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives (62 pages)

860 (1996) Nursing practice
Report of a WHO Expert Committee (37 pages)

861 (1996) Integration of health care delivery
Report of a WHO Study Group (74 pages)

862 (1996) Hypertension control
Report of a WHO Expert Committee (89 pages)

863 (1996) WHO Expert Committee on Specifications for Pharmaceutical Preparations
Thirty-fourth report (200 pages)